THE GROSS MORPHOLOGY AND HISTOCHEMISTRY OF RESPIRATORY MUSCLES IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

Pamela B. Cotten

A Thesis Submitted to the
University of North Carolina Wilmington in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

Department of Biology and Marine Biology

University of North Carolina Wilmington

2007

Approved by

Advisory Committee

_____ _____

_____ _____

_____

Accepted by

_____
Dean, Graduate School

This thesis has been prepared in the style and format

consistent with the journal

Journal of Morphology

TABLE OF CONTENTS

ABSTRACT

Most mammals possess stamina because their locomotor and respiratory (i.e. ventilatory) systems are mechanically coupled. These systems are decoupled, however, in bottlenose dolphins (*Tursiops truncatus*) as they swim on a breath-hold. Locomotion and ventilation appear to be coupled only during their brief surfacing event, when they respire explosively (up to 90% of total lung volume in approximately 0.3s) (Ridgway *et al.*, 1969). The predominantly slow-twitch fiber profile of their diaphragm (Dearolf, 2003) suggests that this muscle does not likely power their rapid ventilatory event. Based upon Bramble's (1989) biomechanical model of locomotor-respiratory coupling in galloping mammals, I hypothesized that muscles located within the cranial-cervical and lumbo-pelvic units, which act upon the thoracic unit, function to power ventilation in bottlenose dolphins. I also hypothesized that these muscles would be composed predominantly of fast-twitch fibers to facilitate the bottlenose dolphin's rapid ventilation. The gross morphology (n=6) of cranio-cervical (sternomastoid, sternohyoid, scalenes), thoracic (intercostals), and lumbo-pelvic (rectus abdominis, abdominal obliques, hypaxialis) muscles and the fiber-type profiles (n=6) of selected muscles (sternohyoid, sternomastoid, and rectus abdominis) of bottlenose dolphins were investigated. Physical manipulations of excised thoracic units were carried out to investigate potential actions of these muscles. Results suggest that the cranio-cervical muscles act to draw the sternum and associated ribs cranio-dorsally, which flares the ribs laterally, and increases thoracic cavity volume required for inspiration. The thoracic muscles physically link the ribs to create a single functional unit; these muscles can also act to control the size of the intercostal space. The lumbo-pelvic muscles act to draw the sternum and caudal ribs

caudally, which decreases the volume of the thoracic and abdominal cavities required for expiration. All muscles investigated were composed predominantly of fast-twitch fibers (range 72-88% by area) and appear histochemically poised for rapid contraction. These combined results suggest that dolphins utilize muscles, similar to those used by galloping mammals, to power their explosive ventilation. However, the mechanisms that permit dolphins to selectively couple and uncouple their locomotor and ventilatory systems, depending upon whether they are respiring at the surface or swimming on a breath-hold, remain unknown.

ACKNOWLEDGMENTS

I would like to thank the following organizations which so graciously provided specimens: the Marine Mammal Stranding Program at the University of North Carolina Wilmington, the Cetacean and Sea Turtle Team at the National Marine Fisheries Service in Beaufort, North Carolina (especially Gretchen Lovewell and Bruce Ferrier), and the Virginia Aquarium Stranding Response Program (especially Sue Barco).

I would like to thank all of the members of the VAB LAB, for all of their assistance, support, and critiques along the way. Special thanks to Marina Piscitelli, whose assistance and inquisitive mind helped me through the long days of dissections and physical manipulations.

I would like to thank my husband, Mike Cotten, for all of his patience and understanding during my time in graduate school. In addition, I would like to thank Blair Harrison whose generosity and hospitality made attending graduate school possible.

I gratefully acknowledge all of my committee members, Drs. Koopman, Kinsey, Dillaman, and Ballard for all of their insightful discussions and time. I would also like to thank William "WAM" McLellan for his creative thinking and thought provoking conversations that formed the fundamental basis of this research and Sentiel "Butch" Rommel for assistance in learning the Easy CAD software; without these two gentlemen this research would not have been possible. And lastly, Ann "DAP" Pabst provided countless hours of guidance, support, enthusiasm and critiques, and I would like to thank her for going above and beyond her duties as a mentor and advisor.

DEDICATION

I would like to dedicate this thesis to my parents, Ann-Marie and Ken Madden, who both have always encouraged me to pursue my passions with their continued love and support.

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

.

INTRODUCTION

Many terrestrial mammals mechanically couple their locomotion and respiration (i.e. ventilation) (Bramble and Carrier, 1983; Bramble, 1989; Alexander, 1993; Bramble and Jenkins, 1993). Bramble (1989) hypothesized that in galloping mammals, muscles located within the cranio-cervical and lumbo-pelvic units, which are typically viewed as locomotor muscles, create the changes in thoracic cavity shape required for inspiration and expiration, respectively. In contrast to terrestrial mammals, bottlenose dolphins (*Tursiops truncatus*) have decoupled locomotion and ventilation, since they swim underwater on a breath-hold. Locomotor–respiratory (i.e. ventilatory) coupling appears to occur only briefly at the surface during their explosive ventilatory event but to date, this mechanical coupling has not been investigated. Using Bramble's (1989) terrestrial mammal locomotor-respiratory model, this study investigated the gross morphology and histochemical profiles of the muscles within the cranio-cervical and lumbo-pelvic units of the bottlenose dolphin, which are in a position to create the thoracic shape change required for ventilation of the lungs.

Terrestrial mammals respire using a costal aspiration pump and a derived diaphragm muscle (reviewed in Brainerd, 1999). Lungs are ventilated by alternating changes in volume and pressure within the thoracic cavity, and thus the lungs, relative to atmospheric pressure. The "textbook" explanation of ventilation in resting mammals describes the diaphragm as the primary muscle of inspiration (reviewed in Schmidt-Nielsen, 1997). The contraction and consequent flattening of the diaphragm increases thoracic cavity volume, which decreases pressure within the lungs, to drive inspiration. Based upon gross morphological and experimental studies, other muscles that can

contribute to inspiration are those that can expand the thoracic cavity by rotating the ribs cranially and laterally. These muscles include the scalenes, transverse thoracic, external and internal intercostals, rectus thoracis, costal levators, cranial dorsal serratus, sternohyoid, and sternomastoid (e.g. Raper et al., 1966; Duron, 1973; De Troyer and Kelly, 1984; Van de Graaff et al., 1984; De Troyer et al., 1985; De Troyer and Ninane, 1986; Nickel et al., 1986; De Troyer, 1991; Whitelaw et al., 1992; Hermanson and Evans, 1993; De Troyer et al., 1994; Carrier, 1996; Fournier and Lewis, 1996; Legrand et al., 1997; De Troyer et al., 2005).

Expiration in terrestrial mammals at rest is largely controlled by passive elastic recoil of the lungs and musculoskeletal elements (reviewed in Nickel et al., 1986; Schmidt-Nielsen, 1997). When active expiration is required, muscles that can rotate the ribs caudally and medially function to decrease the volume within the thoracic and abdominal cavities (Nickel et al., 1986). These muscles include the external and internal intercostals, rectus abdominis, external and internal abdominal obliques, transverse abdominis, caudal dorsal serratus, and rectus thoracis (e.g. De Troyer et al., 1983; De Troyer et al., 1985; Nickel et al., 1986; De Troyer and Ninane, 1987; Gilmartin et al., 1987; De Troyer et al., 1989; Farkas and Schroeder, 1990; Hermanson and Evans, 1993; Deban and Carrier, 2002).

In contrast to mammals at rest, mechanical coupling occurs between the locomotor and respiratory systems in mammals that are actively moving. In galloping mammals, this coupling occurs at a 1:1 ratio (i.e. one stride per breath) (Bramble and Carrier, 1983; Bramble, 1989). This mechanical coupling likely occurs because locomotor movements facilitate ventilation via dorsal and ventral flexions of the body,

and cranio-caudal movements of the abdominal viscera (i.e. the visceral piston) (Bramble and Carrier, 1983; Carrier, 1987). Dorsal body flexion increases the thoracic cavity volume, which decreases internal pressure and assists inspiration (Figure 1A). During dorsal flexion, which is associated with the aerial phase of a locomotor stride, the abdominal viscera are also displaced caudally. Conversely, ventral flexion decreases the thoracic cavity volume, which increases internal pressure to drive expiration (Figure 1B). During ventral flexion, which is associated with the ground phase of a locomotor stride, the abdominal viscera thrusts cranially towards the diaphragm, which further decreases the volume within the thoracic cavity and aids expiration (Bramble and Carrier, 1983; Bramble, 1989; Alexander, 1993; Bramble and Jenkins, 1993). Locomotor stamina, or endurance, is achieved in many terrestrial mammals because they are able to mechanically couple their locomotion and ventilation of the lungs (Carrier, 1987).

Bramble (1989) described a biomechanical model for the integration of the locomotor-respiratory systems in galloping terrestrial mammals. The model's four principal mechanical components are the thoracic unit, the cranio-cervical unit, the lumbo-pelvic unit, and the visceral piston (Figure 2). This biomechanical model suggests that respiration (i.e. ventilation) in galloping mammals is powered by (1) alternate, rhythmic and coordinated muscle contractions of cranio-cervical and lumbo-pelvic units, which both act upon the thoracic unit, and (2) the oscillatory motions of the visceral piston (Bramble, 1989).

According to Bramble's (1989) biomechanical model, inspiration begins during the aerial phase of locomotion and is initiated by muscles within the cranio-cervical unit. These muscles simultaneously depress the cranio-cervical unit and draw the sternum and

Figure 1. Two stages of galloping in the dog showing, (A) dorsal flexion, and (B) ventral flexion (adapted from Nickel et al., 1986).

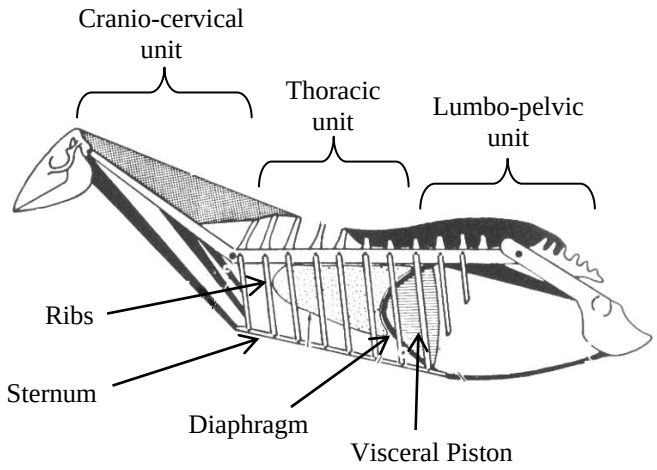


Figure 2. Functional units of the biomechanical model of mammalian locomotor-respiratory coupling (from Bramble, 1989).

ribs cranially, which increases thoracic cavity volume. During this phase, both forelimbs are off the ground and extended cranially, and the lumbo-pelvic unit is extended dorsally, resulting in full dorsal thorax extension. The extension of the body lowers intra-abdominal pressure and causes a caudal displacement of the abdominal viscera (i.e. visceral piston). The combination of lumbo-pelvic unit extension and the rearward displacement of the visceral piston cause an increase in thoracic cavity volume and a decrease in thoracic cavity pressure that draws air into the lungs for inspiration (Bramble, 1989).

Expiration is brought on by the ground reaction forces on the forelimbs, which cause ipsilateral external loading of the thorax and leads to compression of this body cavity, resulting in the reduction of thoracic volume (Bramble, 1989). Additionally, the sudden impact of the forelimbs with the ground results in a cranial displacement of the visceral piston and the diaphragm, which further compresses the thorax and increases the intrathoracic pressure. During this phase, the lumbo-pelvic unit swings cranially due to the contraction of abdominal and axial muscles. Contraction of these muscles further reduces the abdominal volume and raises the internal pressure to assist expiration (Bramble, 1989; Young et al., 1992a). Thus, locomotor movements of the body and concomitant movements of the abdominal viscera facilitate changes in thoracic cavity volume and pressure in galloping terrestrial mammals.

Like terrestrial mammals, bottlenose dolphins (*Tursiops truncatus*), demonstrate locomotor stamina (reviewed in Costa and Williams, 1999); however, locomotor-ventilatory coupling in a dolphin is dependent upon its position within the water column. The majority of a dolphin's life is spent swimming at depth on a breath-hold and as a

result, its locomotion is decoupled from ventilation. Only during surfacing does locomotion and ventilation appear to be mechanically coupled. At the surface, bottlenose dolphins respire explosively and can exchange up to 90% of their total lung volume in approximately 0.3 seconds (Irving et al., 1941; Ridgway et al., 1969; Kooyman and Cornell, 1981; reviewed in Wartzok, 2002). In comparison, a galloping horse exchanges only 21% of its total lung volume in approximately half a second (Hornicke et al., 1983; Young et al., 1992a; Derksen and Robinson, 2002). To date, the mechanisms underlying the bottlenose dolphin's explosive ventilation are not known.

Although the diaphragm is the primary muscle of inspiration in most mammals at rest (reviewed in Brainerd, 1999), its ventilatory role in running mammals is considerably diminished (Bramble, 1989; Alexander, 1993; Bramble and Jenkins, 1993). The diaphragm of bottlenose dolphins has also been hypothesized to play a minor role in ventilation (Dearolf, 2003). Histochemical analysis of the bottlenose dolphin diaphragm revealed that this muscle is composed predominantly of slow-twitch fibers (Dearolf, 2003), suggesting that this muscle may lack the contractile speed required to power explosive ventilation.

Because dolphins live in an aquatic medium they are also not subjected to the abrupt decelerations and reaccelerations of the body experienced by running mammals due to ground reaction forces on the limbs (e.g. Bramble and Jenkins, 1993). Thus, it is unlikely that the visceral piston contributes to bottlenose dolphin ventilation as importantly as it does in terrestrial mammals. Dolphins do, however, undergo cyclical dorso-ventral body flexions, homologous to the axial body movements of galloping mammals, during swimming (e.g. Fish, 1993; Pabst, 1993; Pabst et al., 1999). Bottlenose

dolphins swim by alternate actions of dorsal epaxial, and ventral hypaxial and abdominal muscles (e.g. Slijper, 1936; Arkowitz and Rommel, 1985; Bello et al., 1985; Pabst, 1990, Pabst, 1993; Pabst et al., 1999). These dorso-ventral body movements could, as they do in running mammals, change thoracic and abdominal cavity volumes and pressures.

The bottlenose dolphin thoracic cavity has enhanced flexibility, relative to terrestrial mammals (reviewed by Rommel, 1990), which may contribute to the dolphin's ability to achieve a rapid ventilatory event. In contrast to terrestrial mammals, bottlenose dolphins possess bony sternal ribs that articulate to the distal ends of the vertebral ribs 1 through 7 via synovial joints. This series of "extra" joints has been hypothesized to enhance flexibility to accommodate thoracic compression that occurs during diving (Ridgway et al., 1969; Hui, 1975; Rommel, 1990; Rommel and Reynolds, 2002). To date, the movement of the skeletal elements within the thorax during ventilation has not been investigated or described.

There exist few anatomical descriptions of post-cranial muscles in bottlenose dolphins: Pabst (1990; 1993) described the axial locomotor muscles, Reidenberg and Laitman (1994) described the anatomy and musculature of the hyoid apparatus (in Odontoceti, including *T. truncatus*), and Dearolf (2002) described the morphology of the diaphragm muscle. Although, a small number of anatomical descriptions have been conducted on other cetaceans [e.g. *Balaenoptera acutorostrata* (Carte and Macalister, 1868), *Grampus griseus* (Murie, 1871), *Neophocaena phocaenoides* (Howell, 1927), *Monodon monoceros* (Howell, 1930), *Phocoena phocoena* (Slijper, 1936; Smith et al., 1976), *Pontoporia blainvillei* (Strickler, 1978)], these studies either do not mention or do

not describe in detail muscles used for ventilation. To date, only Dearolf (2002) has examined one ventilatory muscle, the diaphragm, in a bottlenose dolphin.

Bramble's (1989) biomechanical model illustrates that the muscles within the cranio-cervical and lumbo-pelvic units likely facilitate ventilation in galloping mammals. Based upon this model, this study tested the hypothesis that these muscles could also function to power ventilation in bottlenose dolphins. Thus, the goals of this study were two-fold. The first was to describe the gross morphology and identify the potential actions of the muscles located within the bottlenose dolphin's cranio-cervical unit (sternohyoid, sternomastoid, scalenes) and lumbo-pelvic unit (rectus abdominis, abdominal obliques, hypaxialis). Specifically, the muscles located within the cranio-cervical unit were hypothesized to act on the cranial ribs and sternum to increase thoracic cavity volume and create the shape change required for inspiration. The muscles located within the lumbo-pelvic unit were hypothesized to act on the caudal thoracic cavity and sternum to decrease thoracic cavity volume and create the shape change required for expiration. The second goal of this study was to determine the fiber-type profile on a subset of these muscles (sternohyoid, sternomastoid, and rectus abdominis). These muscles were hypothesized to be composed of predominantly fast-twitch fibers to support the bottlenose dolphin's rapid ventilatory event.

MATERIALS AND METHODS

Specimens

Atlantic bottlenose dolphins (*Tursiops truncatus*) that either stranded or were incidentally killed in fishing operations were utilized in this study (Table 1). All

Table 1. Bottlenose dolphin (*Tursiops truncatus*) specimens utilized in this study.

Identification Number	Total Length (cm)	Life History Category	Sex	Code
WAM 606[a,b]	109.0	Neonate	M	2
WAM 610[a,b]	124.5	Neonate	M	3
VAQS 20061008[a,b,c]	175.1	Sub-adult	M	3
BRF 090[a,b]	182.0	Sub-adult	M	3
WAM 627[c]	194.0	Sub-adult	M	2
VAQS 20051086[b,c]	195.4	Sub-adult	M	2
BRF 098[c]	245.0	Adult	F	2
WAM 628[c,*]	246.0	Adult	F	3
BRF 061[a,b,c]	275.0	Adult	M	2
WAM 631[a]	275.0	Adult	F	2

[a]Gross morphology

[b]Physical manipulations

[c]Histochemistry (sternohyoid, sternomastoid, rectus abdominis)

*Regional variation in histochemical profiles

specimens were collected by the Marine Mammal Stranding Program at the University of North Carolina Wilmington, the Cetacean and Sea Turtle Team at the National Marine Fisheries Service Laboratory in Beaufort North Carolina, and the Virginia Aquarium Stranding Response Program. Specimens were placed into life-history categories as defined by Dearolf et al. (2000) and Struntz et al. (2004) and ranged in size from 109cm to 275cm (Table 1). The two smallest specimens (i.e. WAM 606 and WAM 610) were used for preliminary, exploratory dissections; ontogenetic variation was not investigated. Gross dissections were performed on specimens that were in fresh or moderate condition Smithsonian Institution (SI) Code 2 or 3 (Geraci and Lounsbury, 2005). Muscle samples used for histochemical analyses were in fresh condition.

Gross Morphology and Physical Manipulations

The muscles that act upon the thorax were exposed using a routine dissection procedure (outlined in McLellan et al., 2002). Briefly, the blubber layer was carefully flensed off the carcass to expose underlying musculature and connective tissue. The flipper and scapula, their associated intrinsic and extrinsic musculature, and the epaxial muscles were then carefully removed. These steps revealed at least portions of the muscles of interest including the sternohyoid, sternomastoid, scalenes, internal and external intercostals, internal and external abdominal obliques, rectus abdominis, and hypaxialis. The muscles of interest were then carefully exposed and separated along muscle boundaries and/or fascial planes from surrounding musculature. These boundaries were followed along the entire length of each muscle from its origin to its insertion. The gross morphological description of each muscle included a description of

its origin, insertion, fiber orientation, muscle shape, and relationships to other structures, and scaled, digital images were taken throughout each dissection (Nikon D50, JPEG image files).

Muscles photographed *in situ* were imported into a computer engineering drafting software program Easy CAD (Evolution Computing, Phoenix, Arizona) to create representative illustrations of each muscle. To maintain uniformity across all illustrations, each digital image was scaled similarly in Easy CAD and superimposed on a pre-existing, anatomically accurate illustration of a *Tursiops truncatus* skeleton (modified from Rommel, 1990).

Physical manipulations of the thorax were conducted *in situ* (n=6) and on excised thoracic cavity units (n=3). *In situ* manipulations were conducted on partially dissected specimens and the skeletal elements were manually moved in ways to mimic the action of each muscle on the thoracic cavity. Excised thoracic units (thoracic vertebrae, vertebral and sternal ribs, sternum) were mounted on a stable frame, which permitted the thoracic unit to be temporarily fixed in extreme cranial and caudal postures (representing inspiration and expiration, respectively). In these cases, hi-test monofilament fishing line was attached to the cranial (first vertebral rib, first sternal rib, manubrium) and caudal (caudal sternebrae, caudal vertebral ribs) skeletal elements. These lines were manually pulled along trajectories that represented the muscle fiber orientations of the muscles being investigated. A 5kg spring scale (Pensola®, Baar, Switzerland) was used to ensure that repeatable manipulations were achieved by imposing a constant load. Examination of these postures revealed how the muscles located within the cranio-cervical and lumbo-caudal units could create thoracic shape change. Scaled, digital images were taken

during physical manipulations to be analyzed in Easy CAD to examine the changes of skeletal element positions in the two extreme postures. Thoracic skeletal element movement has never been measured in a living bottlenose dolphin. Thus, the postures resulting from these manipulations may underestimate or, more likely, overestimate thoracic shape change that occurs *in vivo*, whereas the manipulations probably do accurately illustrate the direction of skeletal element movements that would occur due to the actions of the muscles within the cranio-cervical and lumbo-pelvic units.

To further model skeletal element movements during a ventilatory event, cervical vertebrae 1-7, thoracic vertebrae 1-10, vertebral and sternal ribs 1-5, and the sternum from an osteological preparation of a bottlenose dolphin were articulated. Small holes were drilled through the body of each vertebra through which a threaded steel rod was inserted and used to align the vertebral column. Thin, small foam pads were placed between each vertebra to represent intervertebral discs and at the proximal and/or distal ends of the bony elements to represent all joints (e.g. vertebra-vertebral rib, vertebral rib-sternal rib, sternal rib-sternum). Small holes were also drilled in each bone and flexible wire was used to articulate the skeletal elements at each joint. The skeletal model was mounted on a stable frame and hi-test monofilament fishing line was attached to skeletal elements to permit manipulations to extreme cranial and caudal postures. The skeletal model was used to identify the movements and rotations of the vertebral and sternal ribs and the movement of the sternum that may occur during ventilation.

Muscle Histochemistry

The histochemical profiles of the sternohyoid (n=6), sternomastoid (n=5), and rectus abdominis (n=6) were investigated (Table 1). Myosin ATPase was used to distinguish between fast and slow contracting muscle fibers. Succinic dehydrogenase (SDH) was used to determine relative oxidative capacity of the muscle fibers. For each muscle, an approximately 3.0cm thick cross-section was sampled from mid-belly at a position equidistant from its origin and insertion. For the rectus abdominis three cross-sectional samples were collected: (1) at a position midway between the origin and the umbilicus (i.e. rectus abdominis-cranial), (2) at the umbilicus (i.e. rectus abdominis-umbilicus), and (3) at a position midway between the umbilicus and its insertion (i.e. rectus abdominis-caudal). To investigate regional variation across the cross-sectional face of each muscle, the fiber-type profile in one adult specimen was sampled at two additional sites. Sternohyoid and sternomastoid muscle samples were obtained from a site on either side of the mid-belly sample. Samples from the rectus abdominis were obtained both medially and laterally from the mid-belly sample. Cross-sectional samples were wrapped in multiple layers of Saran™ Premium Wrap, placed in Ziploc® Freezer bags and frozen flat at -20^{o}C until further analysis (following the methods of Dearolf et al., 2000; Dearolf, 2003; Etnier et al., 2004). For those specimens that were utilized for both physical manipulations and histochemical assays (see Table 1), their sternohyoid, sternomastoid, and rectus abdominis muscles were removed prior to physical manipulations to preserve the quality of the muscle sample.

From each cross section, one small trapezoidal block (<1cm^3) was cut at mid-belly and was permitted to thaw to room temperature (approximately 10 minutes). Once

the muscle blocks were completely thawed, they were placed on a microtome chuck, covered with Optimal Cutting Temperature medium (OCT, Sakura Finetek, Torrance, California) and flash frozen in liquid nitrogen. These frozen muscle blocks were then stored in the microtome (Leica Cryocut 1800, Bunton Instrument Company, Mt. Airy, Maryland) at -19^{o}C for at least two hours prior to cutting, to permit them to warm to a temperature appropriate for thin sectioning. Muscle blocks were serially sectioned (10μm thickness) in the cryostat and mounted on "Plus" glass slides (Fisher Scientific). The "Plus" microscope slides permit tissue sections to electostatically adhere to the glass, decreasing the amount of tissue lost during subsequent staining steps.

Muscle sections were then stained for myosin ATPase. Two slides of serial sections from each muscle block were pre-incubated in acidic (0.200M barbital acetate buffer and 0.100M HCl added to 40mL of deionized water, for a pH of 4.1, 4.3 and 4.4) and alkaline [glycine buffer (74.0mM NaCl and 75.0mM glycine) and 0.150M NaOH added to 31.5mM $CaCl_2$ for a pH of 10.3 and 10.4] mediums. The duration of acidic and alkaline pre-incubation in a 37^{o}C incubator was 5 and 10 minutes, respectively. Acidic and alkaline sections were then incubated in a freshly prepared ATP solution (0.0200M sodium barbital, 17.8mM $CaCl_2$, 2.96mM ATP, for a pH of 9.4) at 37^{o}C for 30 minutes. Sections were then placed in each of the following solutions for three minutes: deionized water, 1% calcium chloride, deionized water, 2% cobalt chloride, deionized water, 1% ammonium sulfide and finally rinsed in deionized water for five minutes. This myosin ATPase protocol follows the methods of Brooke and Kaiser (1970) as modified by Dearolf (2003). Muscle fibers were either classified as type I (slow-twitch) or type II

(fast-twitch) based upon the myosin ATPase acidic and alkaline pre-incubation protocols described in Brooke and Kaiser (1970) and Gauthier (1986).

Additional serial sections were also stained for succinate dehydrogenase to determine relative oxidative potential. The frozen tissue sections warmed to room temperature for 30 minutes prior to being placed in solution [incubating media (equal parts of 0.200M phosphate buffer and 0.193M sodium succinate at pH 7.6, to which 500mL of deionized water was added) and 0.153mM Nitro BT was added] for 60 minutes at 37^{o}C and gently mixed every 5 minutes. The sections were then rinsed in saline solution (16.8mM NaCl) and fixed in a 10% formalin-saline solution. Slides were mounted with Kaiser's glycerol jelly. This succinic dehydrogenase protocol follows the methods of Dearolf et al. (2000) and permitted the muscle fibers to be further subdivided into slow oxidative (type I), fast-twitch oxidative glycolytic (type IIa) and fast-twitch glycolytic (type IIb) based on the intensity of the color stain, which is indicative of their SDH activity.

Images of the sections were viewed using an Olympus Bx60 light microscope and viewed at a magnification of 20x for image analysis. Images were captured using RT KE SPOT camera (Diagnostic Instruments, Inc, version 3.5.6, Sterling Heights, Michigan) and stored as uncompressed images (tagged image format file – TIFF).

Muscle Fiber Analysis

Two methods were used to quantify the muscle fiber profiles. The first method was a stereological approach that determined the area percent occupied by each muscle fiber type (using a Mertz-curvilinear test system) (Bozzola and Russell, 1999; Russ and

Dehoff, 2000). This method provided a more accurate fiber-type profile because it accounts for the differences in size between fibers of different type. The second method was a more traditionally reported count of the number of each fiber type as a percent of the total number of fibers within a prescribed area (Bozzola and Russell, 1999). This method permitted a broader comparison of fiber-type profiles for other species reported in the literature.

The Mertz-curvilinear test system was overlaid on a digital micrograph that was projected on a computer screen and the number of "hits" residing in each fiber type [P_I (the number of hits from a slow-twitch fiber) and P_{II} (number of hits from a fast-twitch fiber)] and white space (P_S) were tallied. White space (P_S) denotes either other structures (e.g. connective tissue, nerve, blood vessels) within the muscle or a separation within the tissue. A "hit" refers to a point that overlays a structure (e.g. the fiber-type that is present at each designated mark on the grid). The number of "hits" counted on SDH treated slides was determined by counting the fibers based on three levels of staining intensity: dark (P_I), intermediate (P_{IIa}), and light (P_{IIb}). This process was repeated for each muscle until a minimum of 500 total fibers were counted. To calculate the area percent occupied by each fiber-type, P_S was subtracted from the total number of possible points (P_T). The resulting value represents the number of hits within muscle tissue (P_M). The percent area for each fiber-type from the myosin ATPase assay was calculated as the fraction (P_I/ P_M) x100 for slow-twitch muscle fibers and (P_{II}/ P_M) x 100 for fast-twitch fibers (Russ and Dehoff, 2000). The percent area for each fiber-type from the succinic dehydrogenase assay was calculated as the fraction (P_I/ P_M) x 100 for slow-twitch oxidative muscle fibers, (P_{IIa}/ P_M) x 100 for fast-twitch oxidative glycolytic muscle fibers,

and (P_{IIb}/ P_M) x 100 for fast-twitch glycolytic muscle fibers. Fiber area percentages were calculated for all three treatments (acid and alkaline pre-incubation and SDH) for each muscle. The precision of percent area values, based upon five repeated measures for each treatment, is ± 2.6%.

The number of fibers of each type, expressed as a percent of the total number of fibers within a prescribed area, was determined by overlaying a 15cm x 15cm grid onto a digital micrograph that was projected on a computer screen. The number of each fiber-type within the grid was counted and this process was repeated until a minimum of 150 total fibers were counted for all three treatments (acid and alkaline pre-incubation and SDH). To compensate for partial muscle fibers that intersect the borders of the grid, only the partial muscle fibers at two of the four borders were counted (Howard and Reed, 1998). To calculate the number of fibers of each type as a percent of the total number of fibers, the total number of each muscle fiber-type [(myosin ATPase: P_I or P_{II}) and (SDH: P_I, P_{IIa}, or P_{IIb})] was divided by the total number of muscle fibers counted (P_m) and multiplied by 100 (Russ and Dehoff, 2000). The precision of the count percent values, based upon five repeated measures for each treatment, is at most ± 1.3%.

Alkaline myosin ATPase stained tissue was used to determine the mean cross-sectional area and mean diameter of individual muscle fibers of ten fibers of each type (fast and slow-twitch) from each muscle. Fibers chosen for analysis had uniform circular cross-sections and were similar in size to surrounding fibers. These fibers were outlined in Photoshop (Adobe Systems, Inc., version 5.0.2), saved as a TIFF file, and analyzed using an imaging analysis software program, Image-Pro® Plus (Media Cybernetics, Inc., version 4.5.0.19, Silver Spring, Maryland).

RESULTS

Gross Morphology and Physical Manipulations

The first goal of this study was to describe the gross morphology and identify the potential actions of the muscles located within the bottlenose dolphin's cranio-cervical unit (sternohyoid, sternomastoid, scalenes) and lumbo-pelvic unit (rectus abdominis, abdominal obliques, hypaxialis). In addition, the external and internal intercostals were described, because they form the muscular wall of the thoracic unit, upon which muscles in the cranio-cervical and lumbo-pelvic units act. Each of the following morphological descriptions includes the origin and insertion of the muscle, its relations to other muscles, its description, and its hypothesized action(s) during ventilation. Because both the origin and insertion of all muscles investigated in this study are moveable, the origin was defined as the cranial end of a muscle. For the abdominal obliques, the origin was defined as the dorsal-most extent of the muscle. The outline of each muscle, and its predominant muscle fiber orientations, are illustrated in the Easy CAD figures and noted after the name of each muscle.

Muscles of the Cranio-Cervical Unit

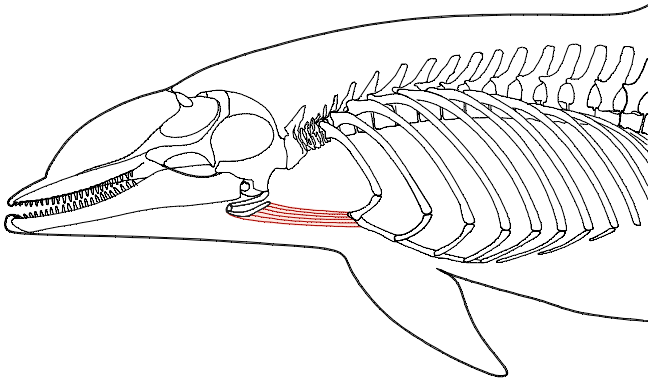
Sternohyoid Muscle (Figures 3A, 3C, 4, 5A)

Origin – Fleshy, off the entire ventral surfaces of basihyal and thyrohyals.

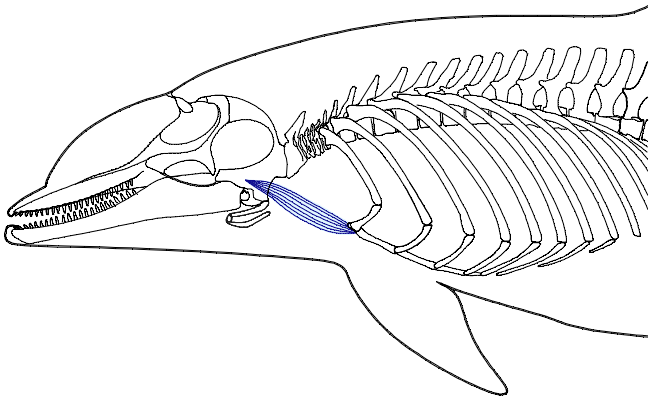
Insertion – Fleshy, onto entire ventral face of manubrium; lateral-most tendons blend with those of the sternomastoid.

Relations – Just deep to blubber layer and superficial to larynx.

A



B



C

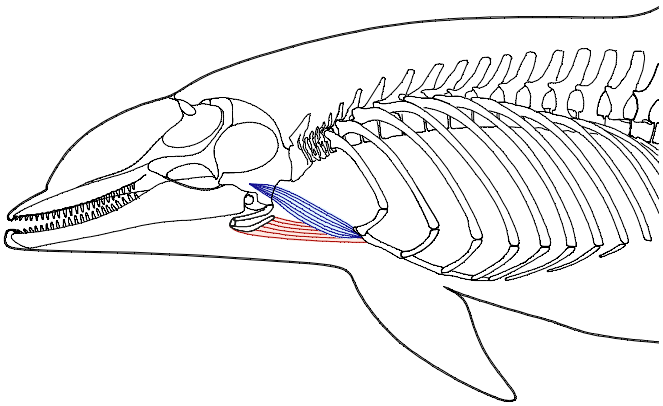


Figure 3. Lateral view of the (A) sternohyoid, (B) sternomastoid, and (C) both sternohyoid and sternomastoid in bottlenose dolphins (*Tursiops truncatus*).

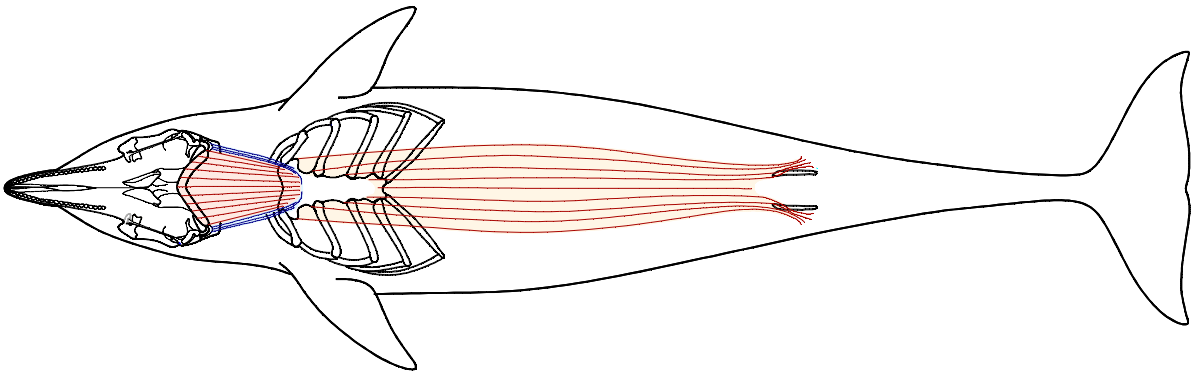


Figure 4. Ventral view of the sternohyoid, sternomastoid, and the rectus abdominis in a bottlenose dolphin (*Tursiops truncatus*).

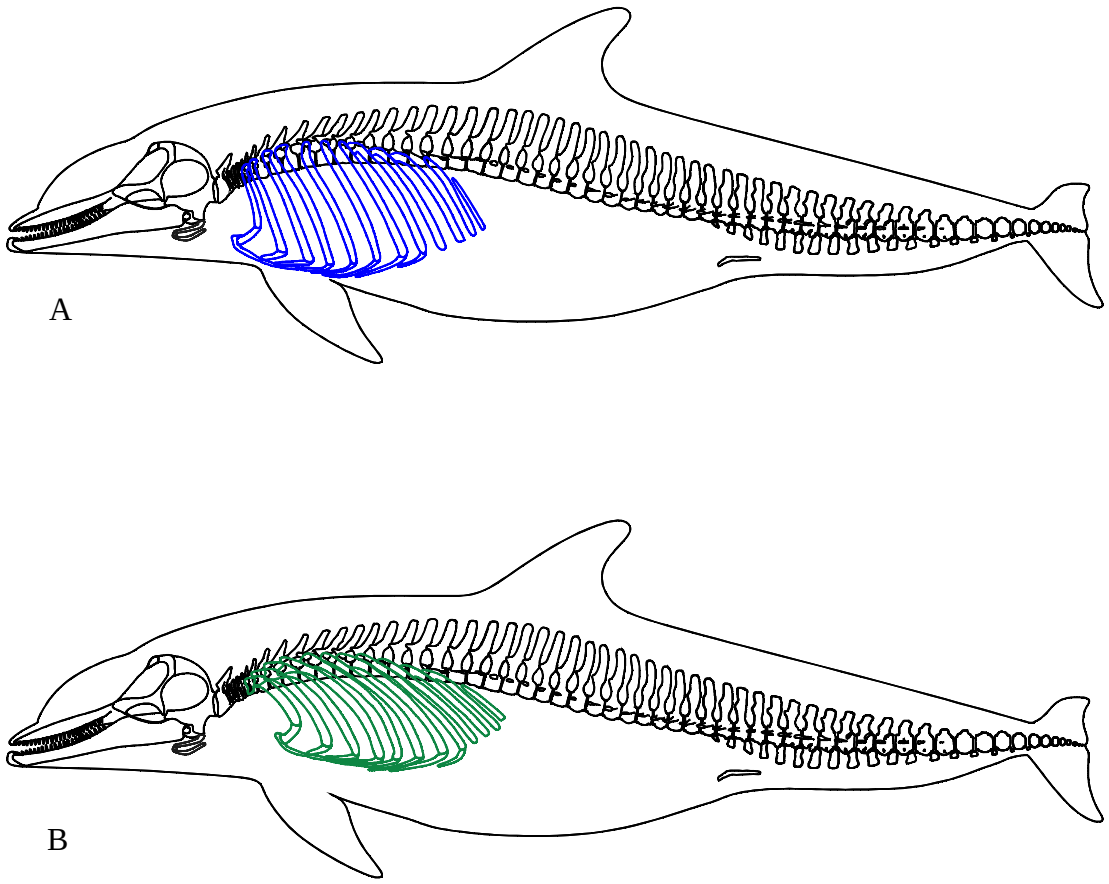


Figure 5. Positions of thoracic skeletal elements at (A) extreme cranial and (B) extreme caudal postures observed during physical manipulations of excised thoracic units. Note that outline of dolphin body does not change.

Description – The sternohyoid is a trapezoidal-shaped muscle that originates broadly and tapers to its more narrow insertion. Its fibers run ventro-caudally from its origin and, near its insertion, blend with those of the sternomastoid.

Action – The action of the sternohyoid depends upon which skeletal element is stabilized because both its origin and insertion are moveable. If the sternum is stabilized, the sternohyoid would draw the hyoid apparatus ventro-caudally. This movement has been hypothesized to function during suction feeding by drawing the tongue ventrally and caudally (e.g. Reidenberg and Laitman, 1994; Heyning and Mead, 1996; Werth, 2000). Alternatively, if the hyoid apparatus is stabilized, the sternohyoid would draw the sternum dorso-cranially. Physical manipulations of the thorax reveal that this movement draws the articulated sternal and vertebral ribs cranially, and causes the vertebral ribs to pivot cranially, relative to the thoracic vertebrae, in two body planes (Figure 5A). The vertebral ribs swing cranially along their length and become more perpendicularly oriented, relative to the long axis of the body. These ribs also pivot cranio-laterally, which causes a lateral flaring of the ribs, within the transverse body plane. The combined movements of the sternum, and the vertebral and sternal ribs, increase thoracic cavity volume, which would aid in inspiration.

<u>Sternomastoid Muscle</u> (Figures 3B, 3C, 4, 5A)

Origin – As a discrete, cylindrically-shaped tendon off the lateral border of the squamosal, just rostral to the tympano-periotic process.

Insertion – As a fan-shaped tendon along entire lateral edge of the ventral face of manubrium. The tendon also extends caudally past the articulation with the first sternal rib to the midline of the second sternebrae.

Relations – At its origin, the sternomastoid lies just ventral and deep to the origins of the mastohumeralis and longissimus capitis. The muscle belly lies just ventral and deep to the thinner mastohumeralis and just superficial to the ventral scalene.

Description – The sternomastoid is a strap-like muscle, with an elliptical cross-section that tapers at both ends. Its fibers run ventro-caudally from its origin.

Action - The action of the sternomastoid depends upon which skeletal element is stabilized because both its origin and insertion are moveable. If the sternum is stabilized, bilateral contraction of the sternomastoid would draw the skull ventrally relative to the atlanto-occipital joint; unilateral contraction would draw the skull laterally. If the skull is stabilized, bilateral contraction of the sternomastoid would cause movements similar to the sternohyoid and therefore aid in inspiration.

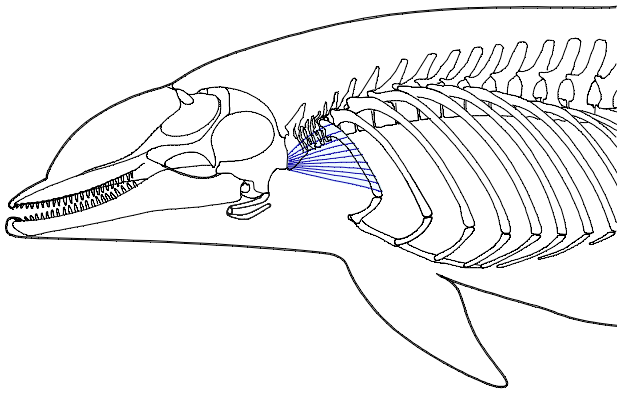
Dorsal Scalene Muscle (Figures 5A, 6A, and 6C)

Origin – Fleshy, off the transverse processes of cervical vertebrae 1-7.

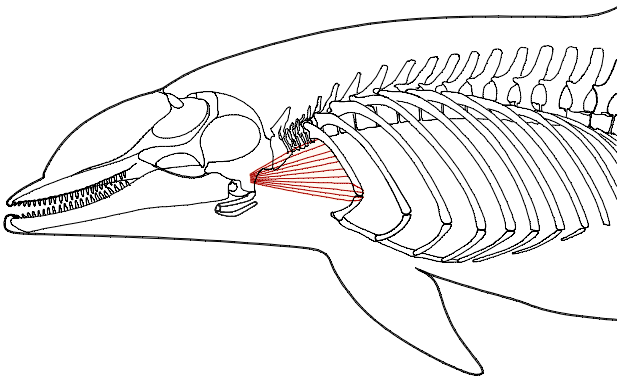
Insertion – Broadly along the lateral edge of the first vertebral rib via two distinct portions. The dorsal-most portion inserts on the rib neck, spanning from the capitulum to the tuberculum. The ventral portion inserts more distally along the entire length of the rib, spanning from the angle to just proximal to the vertebral rib – sternal rib joint.

Relations – The dorsal scalene lies deep to the scapula and its intrinsic musculature and superficial to the dorsal portion of the ventral scalene. At its dorsal margin, it lies just

A



B



C

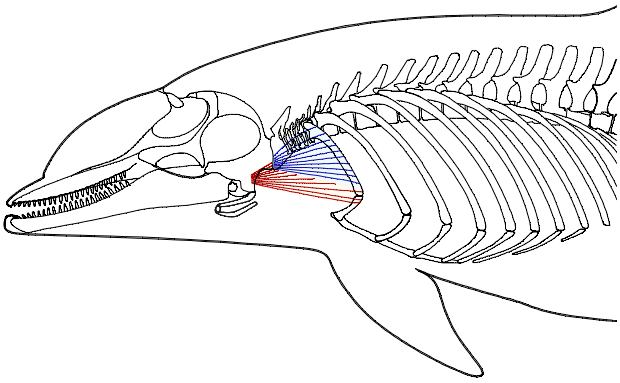


Figure 6. Lateral view of (A) dorsal scalene, (B) ventral scalene, and (C) and both dorsal and ventral scalene in bottlenose dolphins (*Tursiops truncatus*).

ventral to the longissimus capitis. The brachial plexus emerges between the dorsal and ventral scalenes.

Description – Each portion of the dorsal scalene has a distinct shape. The dorsal-most portion is trapezoidal-shaped and its fibers run predominantly longitudinally. The ventral portion is broadly fan-shaped and its fibers run laterally to ventro-caudally to insert along the length of first vertebral rib.

Action – Contraction of the dorsal scalene would draw the first vertebral rib cranially. Physical manipulations of the thorax reveal that this movement causes the first vertebral rib to pivot cranially relative to the thoracic vertebrae, in two body planes (Figure 5A). The first vertebral rib swings cranially along its length and becomes more perpendicularly oriented, relative to the long axis of its body. It also pivots cranio-laterally, which causes it to flare laterally within the transverse body plane. These movements of the first vertebral rib increase thoracic cavity height and width and therefore thoracic cavity volume, which is required for inspiration.

Ventral Scalene Muscle (Figures 5A, 6B, and 6C)

Origin – Mostly fleshy, with short tendons at its ventro-lateral margin, off the ventro-lateral border of the exoccipital.

Insertion – As long, thin tendons along the medial edge of the first vertebral rib spanning from the angle to just proximal to the vertebral rib–sternal rib joint; fleshy, onto a narrow region that spans across the vertebral rib–sternal rib joint and just onto the proximal sternal rib.

Relations –At its origin, the ventral scalene lies caudal to the tympanic bulla and dorsal to the stylohyal articulation with the paroccipital process (Rommel, 1990) of the exoccipital of the skull. The entire muscle lies deep to the scapula and its associated musculature. Its dorsal portion lies deep to the dorsal scalene. Deep to the ventral scalene lies the cranial-most portion of the lung.

Description – The ventral scalene is a broad, fan-shaped muscle, whose fiber orientations vary along its dorso-ventral height. Fibers radiate from the origin and run dorso-caudally to ventro-caudally to its proximal and distal insertions, respectively.

Action – Contraction of the ventral scalene would cause movements of the first vertebral ribs similar to that of the dorsal scalene. Because its insertion crosses the first vertebral rib–sternal rib joint, contraction of the ventral scalene would also directly cause the first sternal rib to pivot cranially. These movements would increase thoracic volume which is required for inspiration.

Muscles of the Thoracic Unit

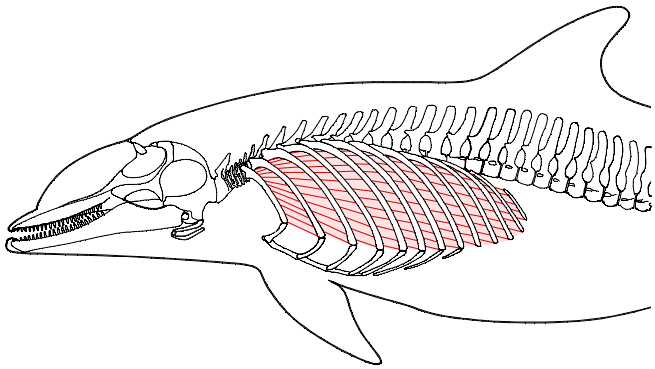
<u>External Intercostal Muscle</u> (Figures 7A and 7C)

Origin – Fleshy, off the entire caudo-lateral border of each vertebral rib.

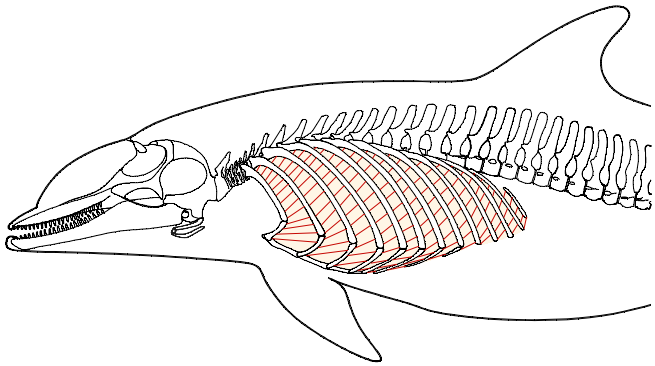
Insertion – Fleshy, and with thin superficial tendons, onto the cranial margin of each caudally adjacent vertebral rib.

Relations – The external intercostals lie superficial to the internal intercostals along the length of the vertebral ribs. The ventral portions of the external intercostals lie deep to the external abdominal oblique muscle.

A



B



C

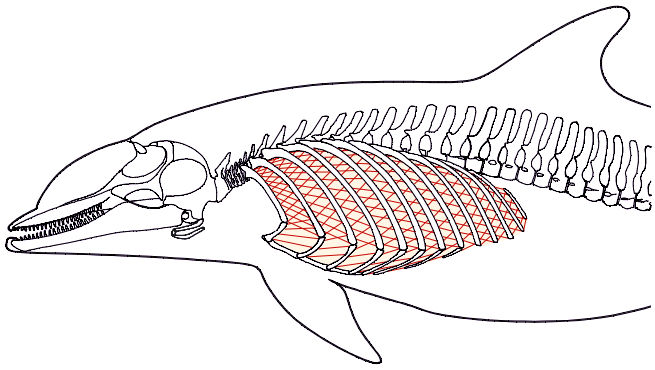


Figure 7. Lateral view of the (A) external intercostals, (B) internal intercostals, and (C) both external and internal intercostals in bottlenose dolphins (*Tursiops truncatus*).

Description – The external intercostals, which connect all serially adjacent vertebral ribs, form the outer muscular layer of the thoracic wall. Between each pair of vertebral ribs, its muscle fibers extend from the tuberculum to the vertebral rib–sternal rib joint. Muscle fiber orientations change along the length of the ribs. At the dorsal-most portion of the external intercostals (i.e. spanning from the tuberculum to the angle of the rib) muscle fibers run longitudinally from their origin. The muscle fibers spanning from the angle of the vertebral ribs to its ventral margin run caudo-ventrally from their origin.

Action – The action of the external intercostals depends upon which skeletal element is stabilized because both its origin and insertion are moveable. The muscle fiber orientation suggests that if the cranial ribs are stabilized, contraction of the external intercostals would draw the caudal ribs dorso-cranially and, therefore, draw the ribs together and assist with inspiration. In contrast, if the caudal ribs are stabilized, contraction of this muscle would draw the cranial ribs ventro-caudally and assist with expiration. If these muscles contract synchronously with the internal intercostals (see below) then they could decrease the intercostal space. Physical manipulations suggested the importance of the external intercostals in mechanically linking the ribs together to create a single functional thoracic unit.

<u>Internal Intercostal Muscle</u> (Figures 7B and 7C)

Origin – As thin, flat tendons along the entire length of the caudo-medial margin of all vertebral and sternal ribs.

Insertion – Fleshy, onto the cranio-medial margin of each caudally adjacent vertebral and sternal rib.

Relations – The internal intercostals lie deep to the external intercostals along the length of the vertebral ribs, and superficial to the transverse thoracic muscle in the ventral thoracic cavity. The cranio-ventral portion of the internal intercostals (between sternal ribs 1-6) lies deep to the external abdominal obliques. Caudal to rib 6, the ventral portion of the internal intercostals lies deep to both the external and internal abdominal obliques.

Description – The internal intercostals, which connect all serially adjacent vertebral and sternal ribs, form the deep muscular layer of the lateral thoracic wall and the superficial layer of the ventral floor of the thorax. Between each pair of ribs, muscle fibers extend from the angle of the vertebral rib to the distal end of all vertebral and sternal ribs. Muscle fibers run dorso-caudally from their origin. The tendons of origin extend approximately halfway across the intercostal space before the muscle becomes fleshy and inserts onto the caudal adjacent rib.

Action – The action of the internal intercostals depends upon which skeletal element is stabilized because both its origin and insertion are moveable. The muscle fiber orientation suggests that if the cranial ribs are stabilized, contraction of the internal intercostals would draw the caudal ribs ventro-cranially and, therefore, draw the ribs together and assist with inspiration. In contrast, if the caudal ribs are stabilized, contraction of this muscle would draw the cranial ribs dorso-caudally and assist with expiration. If these muscles contract synchronously with the external intercostals (see above) then they could decrease the intercostal space. Similar to the external intercostals,

physical manipulations suggested the importance of the internal intercostals in mechanically linking the ribs together to create a single functional thoracic unit.

Muscle of the Lumbo-Pelvic Unit

Rectus Abdominis Muscle (Figures 4, 5B, 8A, 8B, and 8C)

Origin – As thin, flat tendons spanning from the distal half of sternal ribs 1 through 4 to their respective sternal rib–sternum joint. Caudal to sternal rib 4, it becomes fleshy and also originates off the ventral surface of the caudal-most sternebra.

Insertion – Deeply, as a cylindrical tendon onto the cranial margin of the pelvic bone. Superficially as a fan-shaped tendon that crosses the lateral surface of the pelvic bone to join the subdermal connective tissue sheath (Pabst, 1990) before ultimately inserting broadly onto the transverse processes of caudal vertebrae 2 through 7/8.

Relations – The rectus abdominis lies just deep to the blubber layer. At its cranial margin, this muscle lies superficial to the sternum, sternal ribs, and internal intercostals. At its cranio-lateral border, the rectus abdominis lies deep to the pectoralis muscle. Along its length, its lateral edge abuts the ventral margins of the external and internal abdominal obliques. Its superficial and deep surfaces are covered by the rectus connective tissue sheath, formed by the insertional tendons of these abdominal obliques and the transverse abdominal muscle.

Description – The rectus abdominis is a long and robust muscle, with right and left bellies separated by the linea alba. Each belly is deep, broad, and tear-drop shaped in cross-section. From its origin, the muscle progressively deepens and broadens to the

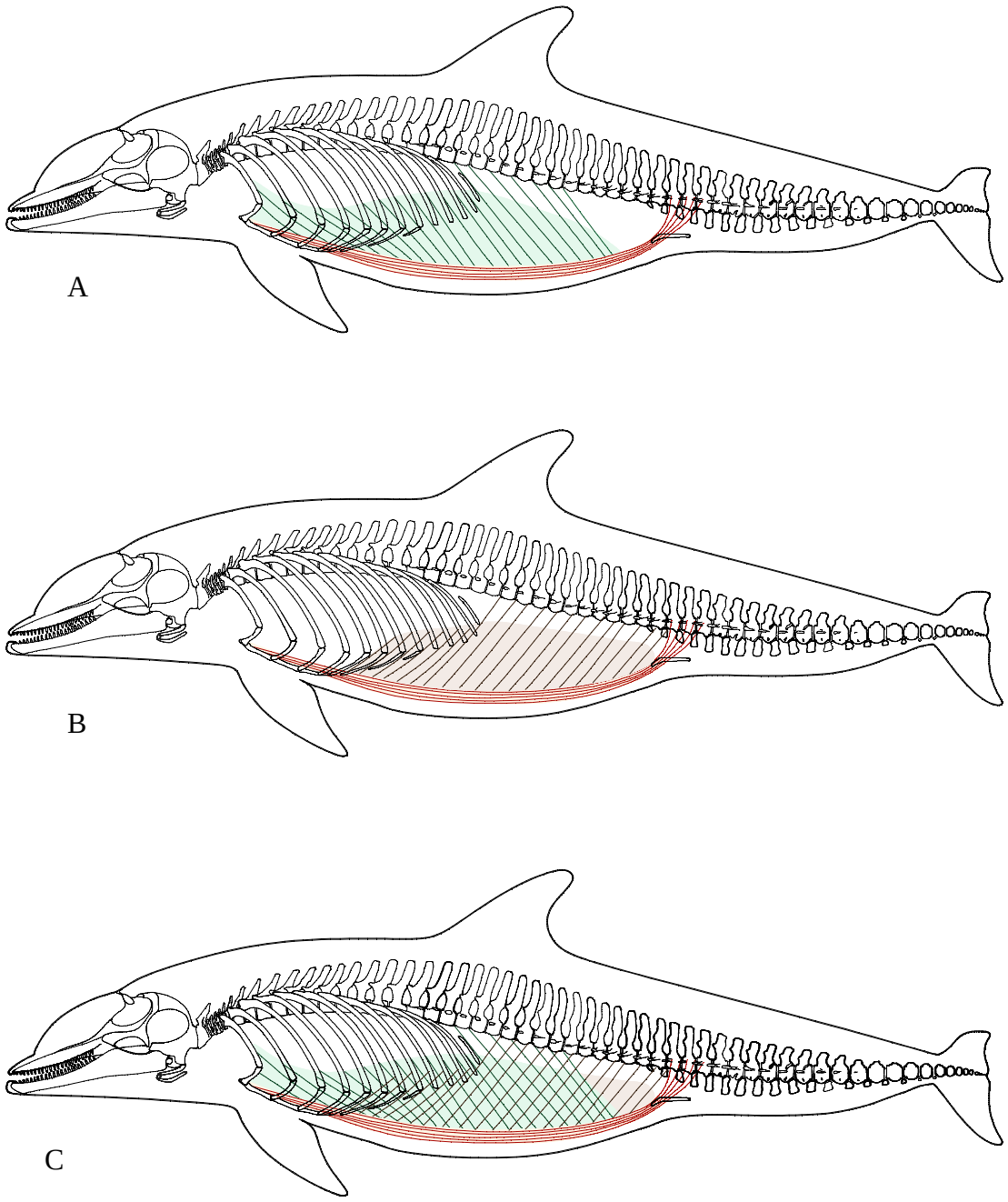


Figure 8. Lateral view of the (A) rectus abdominis and external abdominal obliques, (B) rectus abdominis and internal abdominal obliques, and (C) rectus abdominis, external and internal abdominal obliques in bottlenose dolphins (*Tursiops truncatus*).

level of the umbilicus and then slowly tapers towards its insertion. At its terminal insertion, the bellies separate, and each tapers to a cone before becoming tendinous.

Action - The action of the rectus abdominis depends upon which skeletal element is stabilized because both its origin and insertion are moveable. If the sternum and sternal ribs are stabilized, contraction of the rectus abdominis would draw the pelvic bone and caudal vertebrae (via the subdermal sheath) ventro-cranially. This movement would compress the abdominal cavity and therefore could assist expiration. If the caudal vertebrae are stabilized, contraction of the rectus abdominis would draw the sternal ribs and sternum caudally (Figure 5B). Physical manipulations of the thorax reveal that this movement would also decrease the volume within the thoracic cavity to assist with expiration.

<u>External Abdominal Oblique Muscle</u> (Figures 5B, 8A and 8C)

Origin – Serially, as separate slips off the caudo-lateral border of all vertebral ribs, and occasionally off the superficial surface of the external intercostals. Each slip of the external abdominal oblique arises off a more proximal position on each successively caudal vertebral rib. Its caudal-most origin is off the last vertebral rib and the subdermal connective tissue sheath just caudal to it. The external abdominal oblique arises broadly off the distal-half of the first vertebral rib via robust, flat tendons and via fleshy slips off the proximal first sternal rib. For vertebral ribs 2-6, slips originate via broad flat tendons off the distal one-third of the rib. From vertebral rib 7 caudally, the muscle arises as fleshy slips off the distal portions of the ribs.

Insertion – As an aponeurotic tendon sheet onto the external layer of the rectus connective tissue sheath. These tendons span from the second sternal rib to the level of the caudal-most lumbar vertebrae.

Relations – Across the thoracic wall, the cranio-dorsal most portion of the external abdominal oblique lies superficial to the external intercostals; the cranio-ventral most portions are superficial to the internal intercostals and the pectoralis muscle. Caudal to vertebral rib 7, it lies superficial to the internal abdominal oblique muscle (see below). At vertebral ribs 3 and 4, the slips of the external abdominal oblique interdigitate with those of the serratus ventralis.

Description – The external abdominal oblique is an expansive sheet that covers the ventral portion of the thoracic wall and forms the outer layer of the abdominal wall. Its fibers run ventro-caudally from each slip of origin. Spanning from ribs 1 through 5, a fascial plane exists between the deep surface of this muscle and the superficial surface of the sternal ribs and the internal intercostals; this fascial plane appears to permit separate movement of these layers. Caudal to vertebral rib 5, superficial tendons span across the surface of the muscle and the deep surface is tightly adherent to the superficial surface of the internal abdominal oblique (i.e. no fascial plane exists). Caudal to vertebral rib 10, the superficial tendon fibers appear to replace muscle fibers for much of the length of the external abdominal oblique from its origin to insertion. The muscle originates as fleshy slips but grades into intervening superficial tendons. Just lateral to the rectus abdominis, the muscle becomes fleshy again before its insertion onto the rectus sheath. Thus, much of the external layer of the abdominal wall is formed by tendon, rather than muscle.

Action – If the rectus sheath is stabilized, then the fiber orientation suggests that it would draw the vertebral and sternal ribs ventro-caudally. The distinct fascial plane that exists between the external abdominal obliques and the surface of sternal ribs 1 though 5 suggest that contraction of the external abdominal oblique muscle would decrease the angle of the vertebral rib–sternal rib joints. Physical manipulations of the thorax reveal that the external abdominal obliques can decrease the angle of the vertebral rib–sternal rib joints and aid in rotating the vertebral and sternal ribs caudally. These movements would decrease thoracic and abdominal cavity volume and assist expiration. The tightly adherent connections between the external and internal obliques caudal to rib 6 suggest that no such independent movement of the underlying skeletal elements likely occurs. Simultaneous contractions of both the external and internal abdominal obliques are hypothesized to aid in compression of the thoracic and abdominal cavities.

Internal Abdominal Oblique Muscle (Figures 5B, 8B and 8C)

Origin – Fleshy, off the cranial surface of the distal one-third of caudal vertebral ribs (7-12/13) and the subdermal connective tissue sheath just caudal to the caudal-most rib.

Insertion – Serially, as long, discrete, flattened tendons into the superficial and deep surface of the rectus sheath. These tendons span from the level of the sixth sternal rib to the cranial pole of the pelvic bone and to the level of cranial-most caudal vertebrae.

Relations – Across the thoracic wall, the internal abdominal oblique lies superficial to the external intercostals between the vertebral ribs, and internal intercostals between the caudal-most sternal ribs. Across the abdominal wall, it lies deep to the external abdominal oblique and superficial to the transverse abdominis.

Description – The internal abdominal oblique is an expansive sheet that is completely covered, except at its caudal-most margin, by the external abdominal oblique. Its fibers run ventro-cranially from their origins and cross under those of the external abdominal oblique at approximately right angles. Unlike the external abdominal oblique, muscle fibers traverse most of the distance between its origin and insertion. The superficial surface of this muscle is tightly adherent to the deep surface of the external abdominal obliques.

Action – If the rectus sheath is stabilized, the fiber orientation suggests that it could draw the vertebral and sternal ribs ventro-cranially. The tightly adherent connections between the external and internal abdominal obliques caudal to rib 6 suggest that they form a functional unit that may act simultaneously. Because of the approximate perpendicular fiber orientations of the external and internal abdominal obliques, simultaneous contractions of these muscles are hypothesized to aid in the compression of thoracic and abdominal cavities. Physical manipulations of the thorax revealed that the internal abdominal obliques could decrease the angle of the vertebral rib–sternal ribs joints of the caudal-most ribs. These combined movements would decrease thoracic and abdominal cavity volume to aid expiration.

Hypaxialis Muscle (Figures 5B and 9)

Origin – Fleshy, off the medial surface of vertebral ribs 9-13 and the ventral surface of vertebral bodies from thoracic vertebra 9 to caudal vertebra 18.

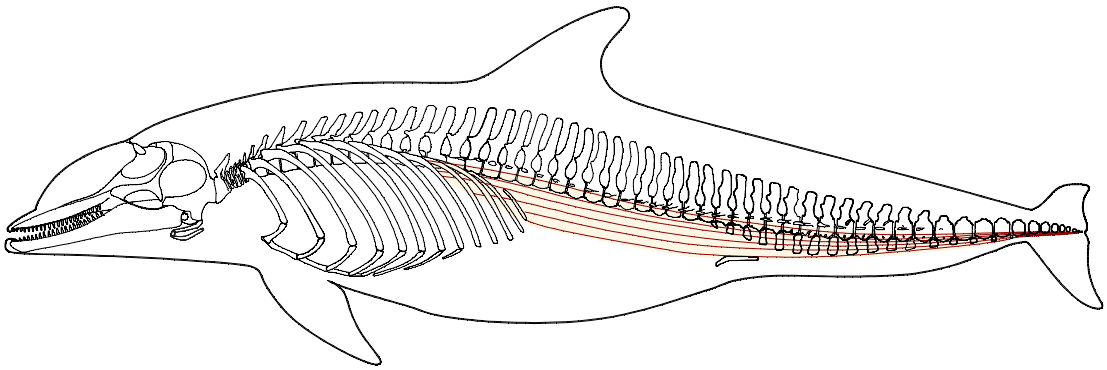


Figure 9. Lateral view of the hypaxialis in bottlenose dolphins (*Tursiops truncatus*).

Insertion – As flattened tendons into the subdermal connective tissue sheath and onto the ventral aspects of vertebrae from mid-lumbar to caudal vertebra 25, including all caudal chevrons.

Relations – The hypaxialis lies deep to ribs 9-13 and ventral to the transverse processes of thoracic, lumbar, and caudal vertebrae.

Description – The long and robust hypaxialis originates as a dorso-ventrally flattened muscle and increases in thickness along its length. At its cranial-most origin (thoracic vertebrae 9 and 10), the hypaxialis spans from the ventral surface of the vertebral body to the vertebra–vertebral rib joints. Between vertebral ribs 11-12, the hypaxialis widens laterally and spans across the proximal third of the caudal-most vertebral ribs. Its fibers run longitudinally from its origin.

Action – The action of the hypaxialis depends upon which skeletal element is stabilized because both its origin and insertion are moveable. If the caudal tailstock is stabilized, contraction of the hypaxialis would draw vertebral ribs 9-13 dorso-caudally (Figure 5B). Physical manipulations of the thorax revealed that these movements would decrease the volume of the thoracic cavity and, thus, aid expiration. If vertebral ribs 9-13 are stabilized, contraction of the hypaxialis would draw the tailstock ventro-cranially. This movement would compress the abdominal cavity and therefore could assist expiration.

Muscle Histochemistry

The alkaline myosin ATPase assay demonstrated that the sternohyoid, sternomastoid, and rectus abdominis each possess a predominantly fast fiber-type profile (range 72.8-88.5% by area) (Table 2; Figure 10A,B). The succinic dehydrogenase assay

Table 2. Mean (± S.E.) percent type II fibers as determined by stereological methods (area) and by direct count, and ratio of type II to type I mean fiber diameters and cross-sectional areas for respiratory muscles of bottlenose dolphins (*Tursiops truncatus*). Values are reported for alkaline myosin ATPase.

Muscle	% Type II Fibers by Area	% Type II Fibers by Count	Diameter II:I	Cross-Sectional Area II:I
Sternohyoid	88.5 ± 1.3	78.9 ± 2.0	1.4	1.8
Sternomastoid	72.8 ± 2.2	57.5 ± 1.7	1.4	2.0
Rectus Abdominis - cranial	77.4 ± 3.7	63.0 ± 4.9	1.5	2.1
Rectus Abdominis - umbilicus	75.6 ± 1.9	58.2 ± 3.5	1.5	2.3
Rectus Abdominis - caudal	74.3 ± 2.0	60.8 ± 2.2	1.3	1.7

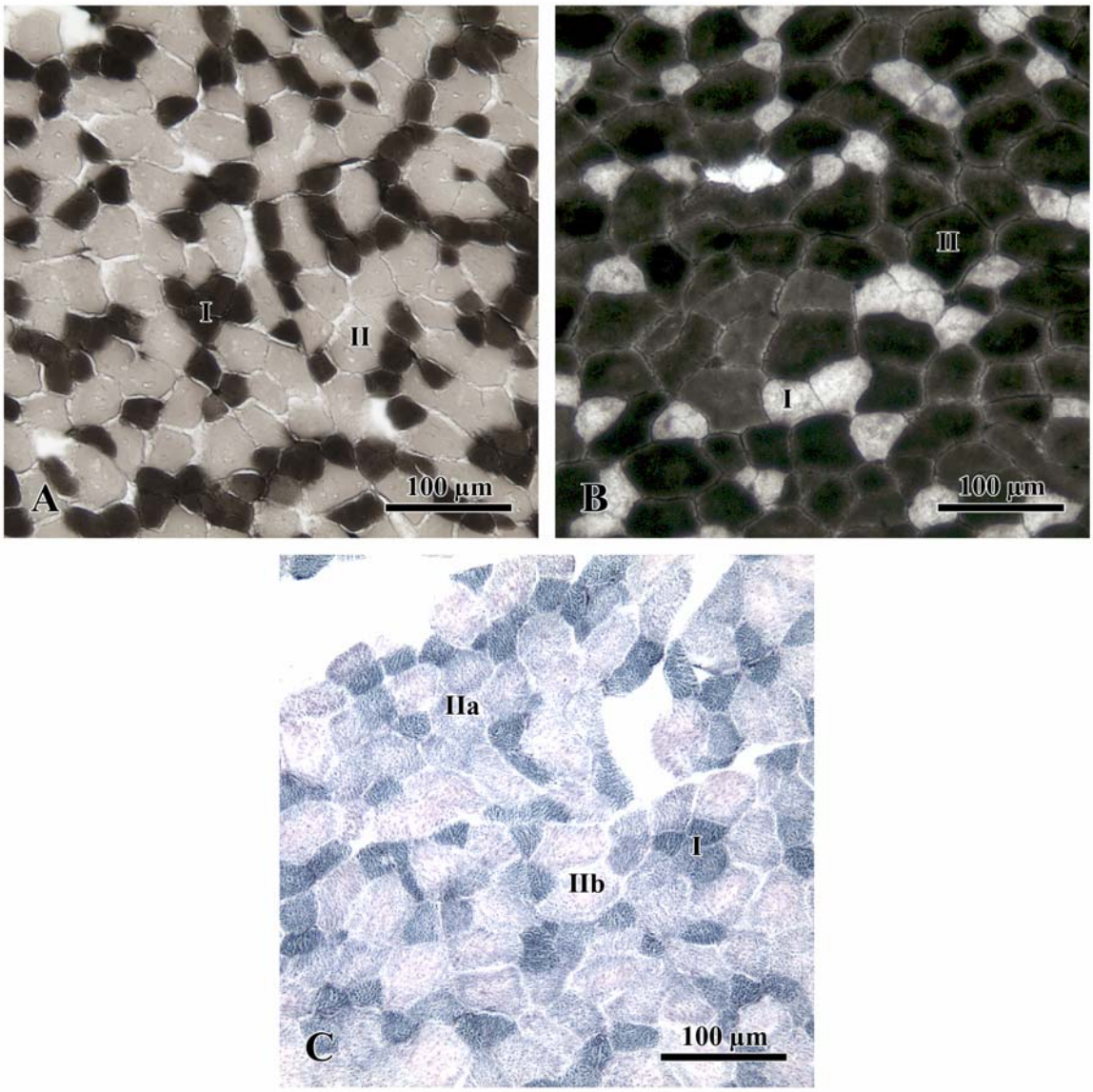


Figure 10. Representative cross-sections of the rectus abdominis muscle in bottlenose dolphins (*Tursiops truncatus*) after histochemical staining: (A) myosin ATPase in acidic pre-incubation solution, (B) myosin ATPase in alkaline pre-incubation solution, and (C) succinic dehydrogenase.

demonstrated three fiber-type populations based upon the staining intensity of the fibers (Figure 10C). The dark staining fibers are type I (slow oxidative), the intermediate staining fibers are type IIa (fast oxidative glycolytic), and the light staining fibers are type IIb (fast glycolytic). The SDH assay demonstrated that the sternohyoid, sternomastoid, and rectus abdominis each possess a predominantly fast-glycolytic profile (Table 3).

For the myosin ATPase assays, the percent fast-twitch (type II) fibers resulting from the stereological approach (i.e. Mertz-curvilinear test system) was always higher (by 10-17%) than that resulting from direct counts (Table 2). Similarly, for the succinic dehydrogenase assay, the percent type IIb fibers resulting from the stereological approach was higher (by 6-12%) than that resulting from direct counts (Table 3). The Mertz-curvilinear test system was a more accurate method than that of the counts because it accounts for the fiber size differences between different fiber-types. Type II fibers were approximately 1.5 times larger in diameter and two times larger in cross-sectional area than type I fibers (Table 2).

The myosin ATPase assay using the alkaline pre-incubation medium resulted in a slightly faster muscle fiber profile (by 3-7%) than the acidic pre-incubation medium for each muscle investigated (Table 4). Samples taken at multiple positions across the muscle's cross-sectional face yielded fiber-type profiles that were similar (differences ranged between 1-7%) to those at the mid-belly (Table 5).

DISCUSSION

Many terrestrial mammals mechanically couple their locomotion and respiration (i.e. ventilation) (Bramble and Carrier, 1983; Bramble, 1989; Bramble and Jenkins,

Table 3. Mean (± S.E.) percent fiber-types determined by stereological methods (area) and direct count, using the succinic dehydrogenase assay for respiratory muscles of bottlenose dolphins (*Tursiops truncatus).*

	% Type I		% Type IIa		% Type IIb	
Muscle	**Area**	**Count**	**Area**	**Count**	**Area**	**Count**
Sternohyoid	28.7 ± 3.0	34.1 ± 1.8	19.9 ± 2.0	20.7 ± 2.0	51.4 ± 2.0	45.2 ± 1.3
Sternomastoid	30.8 ± 4.7	37.5 ± 3.9	17.6 ± 2.8	23.0 ± 2.8	51.6 ± 2.3	39.5 ± 1.8
Rectus Abdominis - cranial	29.2 ± 1.3	35.1 ± 1.3	16.1 ± 1.8	19.7 ± 1.8	54.7 ± 1.5	45.2 ± 1.5
Rectus Abdominis - umbilicus	24.3 ± 4.4	32.5 ± 4.4	16.7 ± 2.2	19.7 ± 2.2	59.1 ± 2.9	47.8 ± 2.9
Rectus Abdominis - caudal	28.7 ± 3.3	40.2 ± 3.3	13.2 ± 2.1	13.2 ± 2.1	58.1 ± 3.7	46.6 ± 3.7

Table 4. Mean (± S.E.) percent type II fibers as determined by stereological methods using alkaline and acidic pre-incubation media for respiratory muscles of bottlenose dolphins (*Tursiops truncatus*).

	% Type II Fibers by Area	
Muscle	**Alkaline**	**Acid**
Sternohyoid	88.5 ± 1.3	87.5 ± 2.0
Sternomastoid	72.8 ± 2.2	68.6 ± 2.4
Rectus Abdominis - cranial	77.4 ± 3.7	69.5 ± 4.7
Rectus Abdominis - umbilicus	75.6 ± 1.9	69.2 ± 3.5
Rectus Abdominis - caudal	74.3 ± 2.0	66.2 ± 2.4

Table 5. Mean (± S.E.) percent type II fibers as determined by stereological methods (area) for variation across the respiratory muscle's cross-sectional face of bottlenose dolphins (*Tursiops truncatus*). For rectus abdominis samples, peripheral site A was a lateral position, and peripheral site B was a medial position. Values are reported for alkaline myosin ATPase.

	% Type II Fibers by Area		
	Sample Site		
Muscle	**Peripheral A**	**Mid-belly**	**Peripheral B**
Sternohyoid	87.7 ± 0.6	88.5 ± 1.3	90.7 ± 0.1
Sternomastoid	73.5 ± 0.1	72.8 ± 2.2	70.3 ± 0.1
Rectus Abdominis - cranial	78.3 ± 0.1	77.4 ± 3.7	80.4 ± 1.0
Rectus Abdominis - umbilicus	82.7 ± 0.8	75.6 ± 2.0	77.7 ± 0.8
Rectus Abdominis - caudal	73.7 ± 0.8	74.3 ± 2.0	75.5 ± 1.0

1993). This mechanical coupling occurs in part because locomotor muscles within the cranio-cervical and lumbo-pelvic units act upon the thoracic unit to facilitate ventilation (Bramble and Carrier, 1983; Bramble, 1989; Bramble and Jenkins, 1993). Using Bramble's (1989) biomechanical model of the integration of locomotor and ventilatory systems in galloping terrestrial mammals, this study investigated the gross morphology and histochemistry of muscles within the cranio-cervical and lumbo-pelvic units in bottlenose dolphins (*Tursiops truncatus*) (Figure 11). This study tested the hypotheses that in bottlenose dolphins these muscles (1) act upon the thorax to change its internal volume to assist with ventilation and (2) are composed of predominantly fast-twitch fibers, to support their rapid ventilation.

Gross Morphology and Physical Manipulations

The muscles investigated within the cranio-cervical unit (e.g. sternohyoid, sternomastoid, dorsal and ventral scalenes) of the bottlenose dolphin are each in a position to increase thoracic cavity volume, which is required for inspiration (Figures 5A and 11). Physical manipulations of excised thoracic units revealed that the sternohyoid and sternomastoid can draw the sternum dorso-cranially, causing the articulated sternal and vertebral ribs to swing cranially and become more perpendicularly oriented relative to the long axis of the body. Contractions of these muscles, and of the scalenes, can pivot the first vertebral and sternal rib cranio-laterally, which also causes lateral flaring of the ribs. Therefore, muscles within the cranio-cervical unit can increase thoracic cavity volume by expanding both its dorso-ventral height and lateral width.

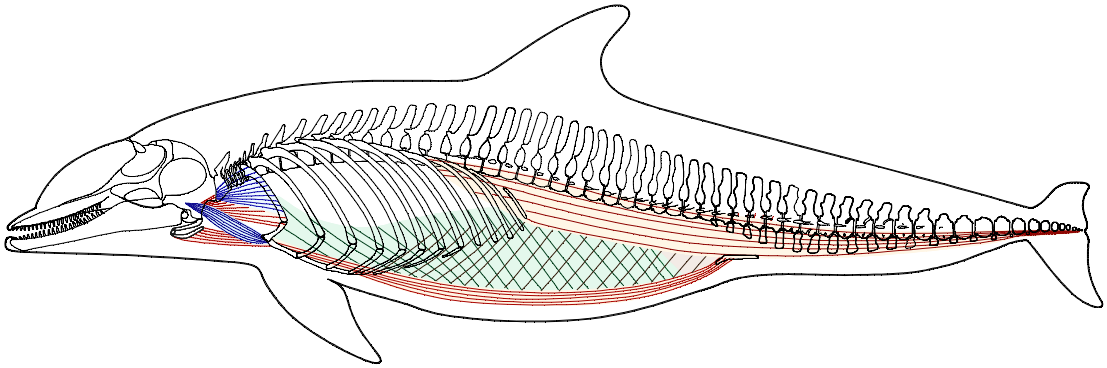


Figure 11. Lateral view of the respiratory muscles within the cranio-cervical and lumbo-pelvic units of bottlenose dolphins (*Tursiops truncatus*).

The results of previous electromyographic studies on terrestrial mammals suggest that cranio-cervical muscles contribute to inspiration, although their relative contributions appear to vary by species and ventilatory activity level. For example, in anesthetized dogs in the supine posture, the sternohyoid displays phasic inspiratory activity (Van de Graaff et al., 1984) and the sternomastoid and scalenes produce lateral expansion of the thoracic cavity that increases lung volume (De Troyer and Kelly, 1984; Legrand et al., 1997). In unanesthetized humans in a seated posture, both the sternomastoid and the scalenes are simultaneously recruited during rapid inspiration, but only the scalenes are active during quiet, resting breathing (Raper et al., 1966). In contrast, the scalene muscle in anesthetized hamsters is inactive during resting ventilation but recruited during rapid ventilation (Fournier and Lewis, 1996). *In toto*, these electromyography studies on terrestrial mammals suggest that the sternohyoid, sternomastoid, and scalenes can contribute to inspiration.

The muscles investigated within the lumbo-pelvic unit (e.g. rectus abdominis, external and internal abdominal obliques, hypaxialis) of bottlenose dolphins are each in a position to decrease thoracic cavity volume, which is required for expiration (Figures 5B and 11). Physical manipulations of excised thoracic units revealed that the hypaxialis draws the caudal-most ribs caudally, decreasing the dorso-ventral height of the thoracic cavity and therefore thoracic cavity volume. The rectus abdominis draws the sternum and the vertebral ribs – via bony articulations with sternal ribs – caudally, also decreasing thoracic cavity dorso-ventral height and volume. This caudal movement of the sternum also appears, though, to cause an increase in the vertebral rib–sternal rib joint angle (Figure 12). The opening of this joint would tend to increase the dorso-ventral height of

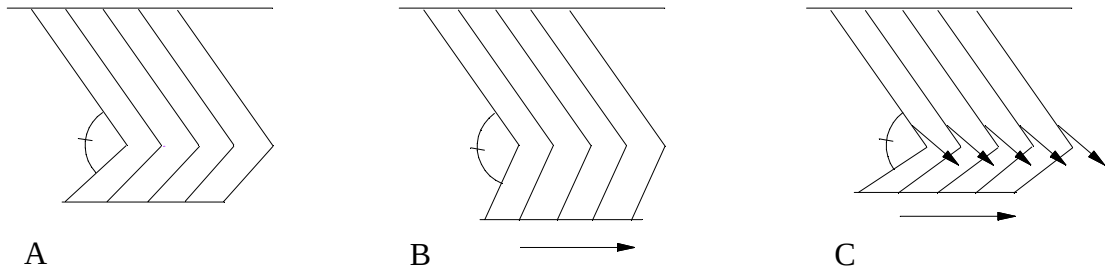


Figure 12. Schematic of the thoracic unit. The top horizontal bar represents the vertebral column and the bottom horizontal bar represents the sternum. The lines at oblique angles represent the vertebral and sternal ribs. (A) is the position of the ribs prior to muscle contraction and, therefore, a “neutral” position, (B) demonstrates the hypothesized angle increase at the vertebral rib–sternal rib joint with caudal movement only from the rectus abdominis, and (C) indicates the hypothesized angle decrease at the vertebral rib–sternal rib joints by caudal movement of the sternum from contraction of the rectus abdominis and the ventro-caudal movement of the ribs caused by the contraction of the external abdominal obliques.

the thoracic cavity, a movement that is contradictory to that of decreasing thoracic cavity volume.

The morphology of the external abdominal oblique muscle suggests that it may act to counter this opening of the vertebral rib-sternal rib joints as the sternum is drawn caudally by the rectus abdominis. Because individual slips of the external abdominal oblique muscle originate off the distal margins of the vertebral ribs and their fibers run ventro-caudally, contraction of this muscle would cause a ventro-caudal movement of the vertebral ribs and a decrease in the angles of the vertebral rib–sternal rib joints (Figure 12). The distinct fascial plane that exists between the superficial surface of sternal ribs 1-5/6 and the deep surface of the external abdominal oblique muscle suggests that these sternal ribs can move freely beneath this muscle. As a result, simultaneous contraction of the external abdominal obliques and the rectus abdominis could decrease the dorso-ventral height of the thoracic cavity and assist with expiration.

In addition, because the external and internal abdominal obliques have fiber orientations approximately perpendicular to each other, simultaneous contraction of these muscles are hypothesized to decrease the dorso-ventral height of the thoracic cavity and aid in compression the abdominal cavity. These movements all contribute to decreasing thoracic and abdominal cavity volumes required for expiration.

Compression of the thoracic and abdominal cavities by muscles within the thoracic and lumbo-pelvic units prior to surfacing could also assist in powering the dolphin's explosive expiration. If the internal and external intercostals contract simultaneously to draw the ribs together the thoracic cavity could be compressed and consequently the pressure would increase. Likewise, if the external and internal

abdominal obliques contract simultaneously to compress the caudal thoracic cavity and the abdominal cavity the pressure would also increase within these cavities. By compressing the volume within the thoracic cavity, and therefore the lungs, prior to surfacing, the resulting increase in pressure could increase the rate of expiratory flow to speed up this phase of the dolphin's ventilatory cycle.

The results of previous electromyographic studies on terrestrial mammals suggest that the lumbo-pelvic muscles do contribute to expiration, although their relative contributions appear to vary by species, posture, and ventilatory activity level. For example, in anesthetized dogs in the supine posture, the rectus abdominis was active during expiration (e.g. De Troyer et al., 1983). Contractions of this muscle cause a decrease in the diameter of the caudal thorax and caudal movement of the sternum and its associated ribs. These movements coincided with an increase in abdominal pressure and a decrease in lung volume (De Troyer et al., 1983; but see Gilmartin et al., 1987 and Farkas and Schroeder, 1990). De Troyer et al. (1983) also found that although the internal abdominal oblique muscle did not cause any cranio-caudal displacement of the sternum and associated ribs, it did cause minimal decrease in the caudal thorax diameter, which coincided with an increase in abdominal pressure. These studies on anesthetized dogs support the hypothesis that the abdominal obliques can aid in abdominal compression and expiration.

In conscious resting dogs the external and internal abdominal obliques were both active during expiration (Deban and Carrier, 2002), whereas when dogs ran, locomotor-ventilatory coupling occurred and the function of the external abdominal oblique muscle varied along its cranio-caudal extent (Deban and Carrier, 2002). The caudal portion of

the external abdominal oblique muscle, spanning over the abdominal cavity, abandoned its ventilatory function. Instead, the activity of this portion of the muscle was associated with the locomotor stride cycle and appeared to function to stabilize the thoracic unit against loads imparted by the limbs (Deban and Carrier, 2002). In contrast, the activity of the external abdominal oblique muscle that covered the thoracic cavity was in phase with both stride cycle and expiration. Thus in running dogs the cranial portion of the external abdominal oblique muscle has a dual role, providing postural support and assisting with expiration (Deban and Carrier, 2002). These results suggested that the potential functions of the abdominal oblique muscles are complex, because they can display regionally specific activities that may vary with animal activity state (De Troyer et al., 1983; De Troyer and Ninane, 1989; Farkas and Schroeder, 1990; Deban and Carrier, 2002). *In toto*, these electromyography studies on terrestrial mammals suggest that the rectus abdominis, abdominal obliques, and hypaxialis can contribute to expiration, as well as play important roles in locomotion.

The results of this study support the hypothesis that muscles within the cranio-cervical and lumbo-pelvic units of the bottlenose dolphin can facilitate inspiration and expiration, respectively. Yet no study to date has investigated the electrical activity of the cranio-cervical or lumbo-pelvic muscles in any cetacean to determine which muscles may be active during ventilation. Electromyographic studies (using non-invasive surface electrodes) would be of value to investigate the role of these muscles in actively respiring bottlenose dolphins.

Although the fiber-type profiles of the investigated muscles are predominantly fast (see discussion below), the explosive ventilation of a bottlenose dolphin occurs so

rapidly that it is unlikely that a complete locomotor sequence can be mechanically coupled to this event. The tail-beat frequency of a dolphin swimming at approximately 2m/s, a typical swimming speed for this species (Williams, 1999), is approximately 1 Hz (Videler and Kamermans, 1985). A full locomotor sequence (upstroke and downstroke) therefore requires at least 1s, but a ventilatory event is completed in only 0.3s (Irving et al., 1941; Ridgway et al., 1969; Kooyman and Cornell, 1981; reviewed in Wartzok, 2002). Therefore, unlike galloping mammals with a 1:1 ratio of breaths per stride, bottlenose dolphins do not require a full locomotor sequence to complete their ventilatory event. The results of this study suggest that when dolphins swim to the surface for a ventilatory event, they alter their typical sequence of locomotion.

A typical locomotor sequence entails a series of downstrokes and upstrokes (Arkowitz and Rommel, 1985; Pabst, 1990; Fish, 1993; Pabst, 1993; Pabst et al., 1999). The downstroke sequence has two portions, (1) the head flexes ventrally via muscles within the cranio-cervical unit (Reidenberg, 2006) and (2) the tail-stock flexes ventrally via the muscles within the lumbo-pelvic unit. The upstroke sequence also has two portions, (1) the head flexes dorsally via cranial epaxial muscles and (2) the tail-stock flexes dorsally via caudal epaxial muscles.

The locomotor muscles within the lumbo-pelvic unit that power the downstroke (Arkowitz and Rommel, 1985; Pabst, 1990; Pabst et al., 1999) also function to decrease thoracic and abdominal cavity volumes (Figures 5B and 11). Thus, expiration is likely mechanically coupled to the downstroke. The morphological results of this study suggest that the ensuing rapid inspiration likely occurs due to simultaneous contractions of the cranial epaxial muscles (the muscles that initiate the upstroke) and the cranio-cervical

muscles. The muscles in the cranio-cervical unit can either (1) draw the sternum and ribs dorso-cranially to increase thoracic cavity volume required for inspiration or (2) ventrally flex the head to initiate the downstroke. To create the movements of the thoracic cavity required for inspiration, the dolphin head must be stabilized. The cranial epaxial muscles could function to stabilize the head so that simultaneous contractions of the cranio-cervical unit muscles could act to increase thoracic cavity volume for inspiration. Therefore, it is hypothesized that in a bottlenose dolphin, a ventilatory event occurs during the terminal portion of the downstroke (via lumbo-pelvic muscles; expiration) and the initial portion of the upstroke (via cranial epaxial muscles). This typical locomotor sequence has superimposed upon it contractions of the cranio-cervical unit (inspiration) simultaneously to those of the cranial epaxial muscles.

In the only published kinematic analysis of surfacing behavior in any cetacean, a wild harbor porpoise (*Phocoena phocoena*) that was enclosed in a fishing weir was observed to approach the surface at an oblique angle and simultaneously ventrally flex its caudal tailstock and dorsally flex its head (Smith et al., 1976). Once the head was above the surface of the water, it was immediately flexed ventrally and inspiration occurred (Smith et al., 1976). Although these rare kinematic data lend partial support for the sequence of muscle activity hypothesized above, they are insufficient to address the ventilatory mechanisms used by cetaceans. Kinematic studies of swimming and respiring bottlenose dolphins are required to provide insight into the distinct portions of their locomotor sequence that are mechanically coupled to their ventilatory event.

In galloping terrestrial mammals, muscle contractions within the cranio-cervical and lumbo-pelvic units create locomotor movements (i.e. dorsal and ventral body

flexions) that facilitate ventilation by changing volume and pressure within the thoracic unit (Bramble and Carrier, 1983; Bramble, 1989; Alexander, 1993; Bramble and Jenkins, 1993). When swimming, bottlenose dolphins also undergo dorso-ventral flexions of the body, which must also affect thoracic cavity volume and pressure, yet they do so on an extended breath-hold. The mechanism that permits bottlenose dolphins to selectively couple and uncouple their locomotion and ventilation remains unknown. However, the results of this study suggest that bottlenose dolphins may be able to differentially stabilize their thoracic cavity during swimming versus surfacing. While swimming on a breath-hold, the thoracic cavity may be relatively more stable, or immoveable, and would therefore, resist shape changes imposed upon it by contractions of muscles within the cranio-cervical and lumbo-pelvic units. In contrast, when a dolphin surfaces for a ventilatory event, the thoracic cavity is dynamic and must undergo shape changes for ventilation.

Results from the physical manipulations on the thoracic unit allowed a new hypothesis to be developed; the variable stability of the thoracic cavity can be achieved by differential actions of the intercostal muscles. When swimming, the intercostal muscles may act as antagonists to the muscles within the cranio-cervical and lumbo-pelvic units. For example, if the intercostal muscles contract to draw the ribs caudally, the thoracic unit would resist deformation caused by muscles in the cranio-cervical unit. In contrast, when surfacing, the intercostal muscles may act as synergist to the muscles within the cranio-cervical and lumbo-pelvic units. For example, if the intercostal muscles contract to draw the ribs cranially, they could facilitate the change in thoracic cavity shape caused by the cranio-cervical muscles. Consequently, the action of the intercostal

muscles could be three-fold: (1) aid in cranial movement of the thorax during contractions of the cranio-cervical muscles, to assist inspiration, (2) aid in caudal movement of the thorax during contractions of the lumbo-pelvic muscles, to assist expiration, and (3) enhance the stability of the whole thoracic unit.

In trotting dogs, brief episodes (up to 2 minutes in length) of uncoupled locomotion and ventilation can occur (Carrier, 1996). These episodes usually occurred during periods when breathing frequency was associated with thermoregulatory transition (i.e. panting) (Carrier, 1996). During these uncoupled periods, the intercostal muscles remained active and their phasic activity was coupled to footfall patterns, not to ventilatory events. Thus, during these periods of decoupled locomotion and ventilation, the intercostal muscles function primarily as locomotor muscles that stabilize the thoracic cavity (Carrier, 1996). These results lend support to the hypothesis that when bottlenose dolphins are swimming, their intercostal muscles may be actively stabilizing the thoracic cavity.

When locomotion and ventilation were mechanically coupled in trotting dogs, the cranial portions of the external intercostal muscle were active during the onset of inspiratory flow and the caudal portion of this muscle and the internal intercostals were active during expiratory flow (Carrier, 1996). The intercostal muscle activity was also phasically locked to the dogs' locomotor stride cycle, which stabilized the thorax against ground reaction forces transmitted by the limbs to the trunk. These results suggest that the intercostal muscles can assist with either ventilation or locomotion, or simultaneously assist both (Carrier, 1996). These results lend support to the hypothesis that when

bottlenose dolphins are surfacing, their intercostal muscles may be active during ventilation.

Electromyographic studies coupled with studies that measure intrathoracic pressure pulses (by a non-invasive technique) in swimming and respiring dolphins, would be of value to address how dolphins could differentially stabilize their thoracic cavity.

The bottlenose dolphin's diaphragm muscle may also contribute to its ability to selectively uncouple locomotion and ventilation. Dearolf (2002) hypothesized that connections between the dolphin diaphragm and the robust ventral connective tissue sheath on the ventral surface of the hypaxialis muscle (Pabst, 1990) prevent the diaphragm from being displaced too far cranially. By limiting the cranial movement of the diaphragm, the cranial movement of the abdominal organs, which likely would occur during ventral flexions of the body, would also be prevented (Dearolf, 2002). Thus, the diaphragm may play an active postural role during locomotion. Dearolf (2002) also hypothesized that during a ventilatory event, the bottlenose dolphin diaphragm may function as an elastic strain energy storage system. The cranial face of the diaphragm is covered by long tendons (i.e. "diaphragmatic ligament"). If these tendons are stretched during expiration, they may be able to recoil elastically to assist with the inspiratory phase. Future studies that investigate the mechanical properties of the tendons within diaphragm of bottlenose dolphins may provide insight into its potential role as an inspiratory spring (Dearolf, 2002).

Muscle Histochemistry

The myosin ATPase assay demonstrated that all muscles investigated within the cranio-cervical and lumbo-pelvic units of bottlenose dolphins were composed predominantly of fast-twitch fibers (Table 2). These fiber-type profiles suggest that each of these muscles is poised for the rapid contractions likely required for their explosive ventilatory event. Interestingly, these dolphin fiber-type profiles are also within the range reported for several terrestrial mammal species that differ in size and locomotor style (Table 6). This shared pattern in fiber-type profiles is likely influenced by a number of factors. For example, the fast muscle fiber profiles observed in smaller species may support their relatively high ventilatory frequencies (Young et al., 1992b). These muscles also play functional roles in multiple body systems. In several families of odontocetes (Kogiidae, Physeteridae, and Ziphiidae), the sternohyoid is hypothesized to be the main contributor to suction feeding (Reidenberg and Laitman, 1994; Heyning and Mead, 1996) and would likely have a fast fiber-type profile to support this rapid kinematic event (Werth, 2000; Bloodworth and Marshall, 2005). However, bottlenose dolphins are primarily ram-based feeders (Bloodworth and Marshall, 2005). This observation suggests that the fast fiber-type profile of the sternohyoid in bottlenose dolphins may more likely contribute to their rapid ventilation than their feeding strategy.

In addition, muscles located within the cranio-cervical and lumbo-pelvic units have traditionally been categorized as locomotor muscles. Young et al. (1992b) suggested that muscle properties are limited by their physiological operations. Fast fiber-type profiles of these muscles may reflect their locomotor as well as ventilatory roles. The dual roles of muscles within the cranio-cervical and lumbo-pelvic units suggest that

Table 6. Mean percent type II fibers of selected muscles in several terrestrial mammal species. Methods used to quantify fiber-types are noted.

	% Type II Fibers				
Animal	**Sternohyoid**	**Sternomastoid**	**Rectus Abdominis**	**Quantification Method**	**Reference**
Dog (*Canis familiaris)*	62.0			Count	Bracher et al., 1997
Dog (*Canis familiaris)*	66.0	79.0	52.0	Count	Armstrong et al., 1982
Opossum *(Didelphis virginiana)*	74.2	74.3	59.7	Count	Hansen et al., 1987
Cat *(Felis catus)*	84.6			Unreported	Dick and van Lunteren, 1990
Hamster *(Mesocricetus auratus)*	94.0			Unreported	Mattson et al., 2002
Rat *(Rattus norvegicus)*	95.0			Count	Bracher et al., 1997
Rat *(Rattus norvegicus)*			80.0	Count	Hijikata et al., 1992
Monkey *(Cebus apella)*			73.0	Unreported	Simionato et al., 2006
Bull *(Bos taurus)*			68.0	Area	Totland and Kryvi, 1991

their profiles should be similar to those of other locomotor muscles. In bottlenose dolphins, two epaxial locomotor muscles, the dorsal intertransversarius and the extensor caudal lateralis, are also composed predominantly of fast-twitch fibers (73.3 and 74.1% by area, respectively) (Dearolf et al., 2000; Etnier et al., 2004). Thus, dolphin locomotor and potential ventilatory muscles have similar fiber-type profiles.

CONCLUSION

This study (1) described the gross morphology of the muscles located within the bottlenose dolphin's cranio-cervical unit (sternohyoid, sternomastoid, scalenes), thoracic unit (intercostals), and lumbo-pelvic unit (rectus abdominis, abdominal obliques, hypaxialis), (2) investigated the potential actions of these muscles through physical manipulations of excised thoracic units and (3) determined the fiber-type profile on a subset of these muscles (sternohyoid, sternomastoid, and rectus abdominis). Results revealed that muscles located within the cranio-cervical unit could create shape changes of the thoracic unit that facilitate inspiration and that muscles within the lumbo-pelvic unit could create shape changes of the thoracic unit that facilitate expiration. It also demonstrated that the fiber-type profiles on a subset of these muscles are composed of predominantly fast-twitch fibers. Similar to Bramble's (1989) biomechanical model of galloping terrestrial mammals, this study found that in the bottlenose dolphin, muscles located with the cranio-cervical and lumbo-pelvic units could facilitate ventilation and that the fast fiber-type profile of these muscles are poised to support this explosive event.

LITERATURE CITED

Alexander RM. 1993. Breathing while trotting. Science 262:196-197.

Arkowitz R, Rommel S. 1985. Force and bending moment of the caudal muscles in the shortfin pilot whale. Marine Mammal Science 1:203-209.

Armstrong R, Saubert C, Seeherman H, Taylor C. 1982. Distribution of fiber types in locomotory muscles of dogs. The American Journal of Anatomy 163:87-98.

Bello M, Roy R, Martin T, Goforth H, Edgerton V. 1985. Axial musculature in the dolphin (*Tursiops truncatus*): some architectural and histochemical characteristics. Marine Mammal Science 1:324-336.

Bloodworth B, Marshall CD. 2005. Feeding kinematics of *Kogia* and *Tursiops* (Odontoceti: Cetacea): characterization of suction and ram feeding. J Exp Biol 208:3721-3730.

Bozzola JJ, Russell LD. 1999. Electron Microscopy: Principles and Techniques for Biologists. Sudbury, MA: Jones and Bartlett Publishers. 670 p.

Bracher A, Coleman R, Schnall R, Oliven A. 1997. Histochemical properties of upper airway muscles: comparison of dilator and nondilator muscles. Eur Respir J 10:990-993.

Brainerd EL. 1999. New perspectives on the evolution of lung ventilation mechanisms in vertebrates. Experimental Biology Online 4:1-28.

Bramble DM. 1989. Axial-appendicular dynamics and the integration of breathing and gait in mammals. Amer Zool 29:171-186.

Bramble DM, Carrier DR. 1983. Running and breathing in mammals. Science 219:251-256.

Bramble DM, Jenkins F Jr. 1993. Mammalian locomotor-respiratory integration: implications for diaphragmatic and pulmonary design. Science 262:235-240.

Brooke M, Kaiser K. 1970. Muscle fiber types: how many and what kind? Arch Neurol 23:369-379.

Carrier DR. 1987. The evolution of locomotor stamina in tetrapods: circumventing a mechanical constraint. Paleobiology 13:326-341.

Carrier DR. 1996. Function of the intercostal muscles in trotting dogs: ventilation or locomotion? J Exp Biol 199:1455-1465.

Carte A, Macalister A. 1868. On the anatomy of *Balaenoptera rostrata*. Philosphical Transactions of the Royal Society of London 158:201-261.

Costa DP, Williams TM. 1999. Marine Mammal Energetics. In: Reynolds JE, Rommel SA, editors. Biology of Marine Mammals. Washington: Smithsonian Institution Press. p 176-217.

Dearolf JL. 2002. Morphology and development of the diaphragm of bottlenose dolphins (*Tursiops truncatus*). PhD thesis, Ithaca: Cornell University.

Dearolf JL. 2003. Diaphragm muscle development in bottlenose dolphins *Tursiops truncatus*. J Morphol 256:79-88.

Dearolf JL, McLellan WA, Dillaman RM, Frierson DJ, Pabst D. 2000. Precocial development of axial locomotor muscle in bottlenose dolphins *Tursiops truncatus*. J Morphol 244:203-215.

Deban SM, Carrier DR. 2002. Hypaxial muscle activity during running and breathing in dogs. J Exp Biol 205:1953-1967.

Derksen FJ, Robinson NE. 2002. Overview of the equine respiratory system. In: Lekeux P, editor. Equine Respiratory Diseases. Ithaca: International Veterinary Information Service.

De Troyer A. 1991. Inspiratory elevation of the ribs in the dog: primary role of the parasternals. J Appl Physiol 70:1447-1455.

De Troyer A, Sampson M, Sigrist S, Kelly S. 1983. How the abdominal muscles act on the rib cage. J Appl Physiol 54:465-469.

De Troyer A, Kelly S. 1984. Action of neck accessory muscles on rib cage in dogs. J Appl Physiol 56:326-332.

De Troyer A, Kelly S, Macklem PT, Zin WA. 1985. Mechanics of intercostal space and actions of external and internal intercostal muscles. J Clin Invest 75:850-857.

De Troyer A, Ninane V. 1986. Triangularis sterni: a primary muscle of breathing in the dog. J Appl Physiol 60:14-21.

De Troyer A, Ninane V. 1987. Effect of posture on expiratory muscle use during breathing in the dog. Respir Physiol 67:311-322.

De Troyer A, Gilmartin JJ, Ninane V. 1989. Abdominal muscle use during breathing in unanesthetized dogs. J Appl Physiol 66:20-27.

De Troyer A, Cappello M, Brichant JF. 1994. Do canine scalene and sternomastoid muscles play a role in breathing? J Appl Physiol 76:242-252.

De Troyer A, Kirkwood P, Wilson T. 2005. Respiratory action of the intercostal muscles. Physiol Rev 85:717-756.

Dick TE, van Lunteren E. 1990. Fiber subtype distribution of pharyngeal dilator muscles and diaphragm in the cat. J Appl Physiol 68:2237-2240.

Duron B. 1973. Postural and ventilatory functions of intercostal muscles. Acta Neurobiol 33:355-380.

Etnier SA, Dearolf JL, McLellan WA, Pabst DA. 2004. Postural role of lateral axial muscles in developing bottlenose dolphins (*Tursiops truncatus*). Proceedings of the Royal Society B: Biological Sciences 271:909-918.

Farkas GA, Schroeder MA. 1990. Mechanical role of expiratory muscles during breathing in prone anesthetized dogs. J Appl Physiol 69:2137-2142.

Fish FE. 1993. Power output and propulsive efficiency of swimming bottlenose dolphins (*Tursiops truncatus*). J Exp Biol 185:179-193.

Fournier M, Lewis MI. 1996. Functional role and structure of the scalene: an accessory inspiratory muscle in hamster. J Appl Physiol 81:2436-2444.

Gauthier GF. 1986. Skeletal muscle fiber types. In: Engel A, Banker B, editors. Myology: McGraw-Hill. p 255-283.

Geraci JR, Lounsbury VJ. 2005. Marine Mammals Ashore: A Field Guide for Strandings. Baltimore, MD: National Aquarium in Baltimore.

Gilmartin JJ, Ninane V, De Troyer A. 1987. Abdominal muscle use during breathing in the anesthetized dog. Respir Physiol 70:159-171.

Hansen S, Cutts JH, Krause WJ, Cutts JH. 1987. Distribution of fibre types in thirty-seven muscles of *Didelphis virginiana*. Anat Anz 164:153-158.

Hermanson J, Evans H. 1993. The muscular system. Miller's Anatomy of the Dog. Philadelphia: W.B. Saunders Company. p 258-384.

Hijikata T, Wakisaka H, Yohro T. 1992. Architectural design, fiber-type composition, and innervation of the rat rectus abdominis muscle. The Anatomical Record 234:500-512.

Hornicke H, Meixner R, Pollmann U. 1982. Respiration in exercising horses. In: D.H. Snow D, Persson S, Rose R, editors. Equine exercise physiology. Cambridge: Granta Editions. p 7-16.

Howell AB. 1927. Contribution to the anatomy of the chinese finless porpoise, *Neomeris phocaenoides*. Proceedings US National Museum 70:1-43.

Howell AB. 1930. Myology of the narwhal (*Monodon monoceros*). The Americal Journal of Anatomy 46:187-215.

Hui, CA. 1975. Thoracic collapse as affected by the retia thoracica in the dolphin. Respir Physiol 25:63-70.

Irving L, Scholander P, Grinnell S. 1941. The respiration of the porpoise, *Tursiops truncatus*. Journal of Cellular and Comparative Physiology 17:145-168.

Kooyman G, Cornell L. 1981. Flow properties of expiration and inspiration in a trained bottle-nosed porpoise. Physiol Zool 54:55-61.

Legrand A, Ninane V, De Troyer A. 1997. Mechanical advantage of sternomastoid and scalene muscles in dogs. J Appl Physiol 82:1517-1522.

Mattson J, Miller T, Poole D, Delp M. 2002. Fiber composition and oxidative capacity of hamster skeletal muscle. The Journal of Histochemistry and Cytochemistry 50:1685-1692.

McLellan WA, Koopman HN, Rommel SA, Read AJ, Potter CW, Nicolas JR, Westgate AJ, Pabst DA. 2002. Ontogenetic allometry and body composition of harbour

porpoises (*Phocoena phocoena*, L.) from the western North Atlantic. Journal of Zoology 257:457-471.

Murie J. 1871. On Risso's grampus, *G. rissoanus* (Desm.). J Anat Physiol 5:118-138.

Nickel R, Schummer A, Seiferle E, Wilkens H, Wille K, Frewein J. 1986. The anatomy of the domestic animals. New York: Springer-Verlag. 449 p.

Pabst DA. 1990. Axial muscles and connective tissues of the bottlenose dolphin. In: Leatherwood S, Reeves RR, editors. The Bottlenose Dolphin. San Diego: Academic Press. p 51-67.

Pabst DA. 1993. Intramuscular morphology and tendon geometry of the epaxial swimming muscles of dolphins. Journal of Zoology 230:159-176.

Pabst DA, Rommel S, McLellan WA. 1999. The functional morphology of marine mammals. In: Reynolds J, Rommel S, editors. Biology of Marine Mammals. Washington: Smithsonian Institution Press. p 35-37.

Raper AJ, Thompson WT Jr., Shapiro W, Patterson JL Jr. 1966. Scalene and sternomastoid muscle function. J Appl Physiol 21:497-502.

Reidenberg JS. 2006. Motor mouth moby: is the hyoid a locomotor bone in whales, dolphins, and porpoises [abstract]? In: American Association of Anatomist. A845-d.

Reidenberg JS, Laitman JT. 1994. Anatomy of the hyoid apparatus in odontoceli (toothed whales): specializations of their skeleton and musculature compared with those of terrestrial mammals. The Anatomical Record 240:598-624.

Ridgway SH, Scronce BL, Kanwisher J. 1969. Respiration and deep diving in the bottlenose porpoise. Science 166:1651-1654.

Rommel S. 1990. Osteology of the bottlenose dolphin. In: Leatherwood S, Reeves RR, editors. The Bottlenose Dolphin. San Diego: Academic Press, Inc. p 29-49.

Rommel SA, Reynolds JE. 2002. Skeletal Anatomy. In: Perrin W, Wursig B, Thewissen J, editors. Encyclopedia of Marine Mammals. San Diego: Academic Press. p 1089-1103.

Russ JC, Dehoff RT. 2000. Practical Stereology. New York: Plenum Press. 185 p.

Schmidt-Nielsen K. 1997. Respiration. Animal physiology: adaptation and environment. Cambridge: Cambridge University Press. p 5-64.

Simionato LH, Andreo JC, de Oliveira JA, Bortoluci CH, dos Santos NB, Moraes LH, Rodrigues AC, Andreo MB. 2006. Morphometry and histochemistry of the rectus abdominis muscle fibers of tufted capuchin monkeys (*Cebus apella* Linnaeus, 1758). International Journal of Morphology 24:53-60.

Slijper EJ. 1936. Die Cetaceen, vergleichend-anatomisch und systematisch. *Capita Zool Bd VI and VII* 7:1-600.

Smith G, Browne K, Gaskin D. 1976. Functional myology of the harbour porpoise, *Phocoena phocoena*. Can J Zool 54:716-729.

Strickler T. 1978. Myology of the shoulder of *Pontoporia blainvillei*, including a review of the literature on shoulder morphology in the Cetacea. The Americal Journal of Anatomy 152:419-431.

Struntz DJ, McLellan WA, Dillaman RM, Blum JE, Kucklick JR, Pabst DA. 2004. Blubber development in bottlenose dolphins (*Tursiops truncatus*). J Morphol 259:7-20.

Totland G, Kryvi H. 1991. Distribution patterns of muscle fiber types in major muscles of the bull (*Bos taurus*). Anat Embryol 184:441-450.

Van de Graaff WB, Gottfried SB, Mitra J, van Lunteren E, Cherniack NS, Strohl KP. 1984. Respiratory function of hyoid muscles and hyoid arch. J Appl Physiol 57:197-204.

Videler J, Kamermans P. 1985. Differences between upstroke and downstroke in swimming dolphins. J Exp Biol 119:265-274.

Wartzok D. 2002. Breathing. In: Perrin W, Würsig B, Thewissen H, editors. Encyclopedia of Marine Mammals. San Diego: Academic Press. p 164-168.

Werth A. 2000. A kinematic study of suction feeding and associated behavior in the long-finned pilot whale, *Globicephala melas* (traill). Marine Mammal Science 16:299-314.

Whitelaw WA, Ford GT, Rimmer KP, De Troyer A. 1992. Intercostal muscles are used during rotation of the thorax in humans. J Appl Physiol 72:1940-1944.

Williams TM. 1999. The evolution of cost-efficient swimming in marine mammals: limits to energetic optimization. Phil Trans R Soc Lond B 354:193-201.

Young I, Alexander R, Woakes A, Butler P, Anderson L. 1992a. The synchronization of ventilation and locomotion in horses (*Equus caballus*). J Exp Biol 166:19-31.

Young IS, Warren RD, Altringham JD. 1992b. Some properties of the mammalian locomotory and respiratory systems in relation to body mass. J Exp Biol 164:283-294.